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MANUAL OF LABORATORY DIAGNOSIS

GARDNER AND LINCOLN

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MANUAL

Of

Laboratory Diagnosis

By

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PREFACE.

The material in this manual has as its basis the lecture notes and outlines prepared by us and used during the past eight years in teaching the subject of Laboratory Diagnosis.

It has been our purpose first and chiefly to give practical working directions for making the important clinical laboratory tests, and, second, to give the clinical significance of the findings.

It is published in the hope that it may be of service to students of medicine and to physicians who do their own laboratory work or who want assistance in the interpretation of laboratory findings. It is believed, also, that it may be found useful to trained nurses and to technicians in clinical laboratories.

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CHAPTER I.

BLOOD.

The routine clinical examination of the blood consists in:

1. Estimation of the haemoglobin.
2. Counting the erythrocytes.
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4. Examination of the stained film.

Obtaining the Blood for Examination.—

A sharp blood lance is needed, one with a spear-shaped point being preferable. A Hagedorn needle, such as is used for puncturing ear drums, makes an excellent blood lance.

The front of the lobe of the ear is the best place to obtain the blood. The ear is less sensitive than the finger and is out of the patient's sight, a good thing in cases of nervous children or persons likely to faint at the sight of blood. The site of the puncture and the lance are cleansed with alcohol. A quick stab is less painful than a punching motion. The stab should be deep enough to secure a drop of blood without much squeezing.

Estimation of the Haemoglobin.—

None of the methods simple enough for common use is accurate, but changes in haemoglobin sufficient to have clinical significance will be shown by any of them. The Tallquist method gives as good results as the Dare or the Sahli, except in cases in which the haemoglobin is below

40 per cent. Below this point it is not easy to match the color scale.

The Tallquist haemoglobin scale consists of a book containing sheets of absorbent paper and a color scale. A sheet of the absorbent paper is touched to a drop of blood large enough to make a spot on the paper greater in circumference than the round openings in the color scale. When the blood has soaked in but not dried, the blood spot is placed behind the different openings in the color scale until a match is found. In order to secure a white background for the blood drop the absorbent paper is folded back of the spot, and the color scale with the test paper is pressed flat against the cover of the book.

Theoretically 100 per cent represents the normal amount of haemoglobin, but many well persons have haemoglobin as low as 80 per cent and not many city dwellers have above 90 per cent.

Counting the Corpuscles.—

The Thoma-Zeiss Haemocytometer consists of two pipettes, one for erythrocytes, one for leucocytes; and a counting chamber, which is formed by a slide with a ruled space, and a cover glass. The counting chamber with the Türck ruling is preferred.

Counting Erythrocytes.—

The red cell pipette has the mark 101 above the bulb. For counting erythrocytes a diluting fluid which will preserve red cells is used. Physiological salt solution may be used, but Hayem's fluid is better.

Hayem's diluting fluid for erythrocytes:

Sodium Chloride, 1 gram.
Sodium Sulphate, 5 grams.
Mercuric Chloride, 0.5 gram.
Distilled Water, 200 c.c.

The pipette is touched to a freshly exuded drop and the blood is drawn up to the mark 0.5. Any excess of blood is wiped from the end of the pipette, and the pipette is immersed in the diluting fluid, which is drawn up to the mark 101, the pipette being rotated the while to mix the fluids. Both ends of the pipette are closed with thumb and finger and the pipette is shaken until blood and diluting fluid are thoroughly mixed. Two or three drops are then blown out and a drop is mounted for counting.

The counting chamber consists of a slide upon which is cemented a circular piece of glass, the island. Upon the surface of the island one square millimeter is ruled into 400 small squares. If the counting chamber has the Türck ruling, this square millimeter is surrounded by eight other squares of equal size, with a few intersecting lines in each square.

Surrounding the island and also cemented to the slide is a square of glass with a circular hole in the middle large enough to encircle the island, leaving a moat or ditch between. This square is 0.1 millimeter thicker than the island, so that the counting chamber formed when the cover glass is placed is 0.1 millimeter deep.

When ready to mount the drop for counting, the end of the pipette is wiped, its point brought in contact with the island at an angle of 45 degrees and a drop milked out by pressing with the fingers on the rubber tubing attached to the pipette. The size of the drop can be well controlled in this way. The drop must be just large enough to fill the island and not run over into the moat when the cover glass is applied.

To place the cover glass, take it by one corner and let the opposite corner touch the slide at the side of the square, so that the cover glass leans against the edge of the square and is held in this position by the finger. Steadied in this way,

it can be settled into place without forming bubbles in the counting chamber.

Allow a few moments for the corpuscles to settle, then place the slide under the microscope and take a general view of the field with the low-power lens to see if the corpuscles are evenly distributed. If they are not even, another mount must be made. If they are, the preparation is ready to count. A high dry lens is usually used for counting. An arrangement of lens and eye piece is desirable which will bring sixteen small squares into the field at the same time. It will be seen that every fifth small square is intersected by a line drawn through the middle, thus, blocks of sixteen small squares, none of which have intersecting lines, are marked off.

Count all the corpuscles in five of these blocks of sixteen and find their sum. To this sum add four ciphers and the result is the number of corpuscles per cubic m.m.

In counting five blocks of sixteen, eighty small squares have been counted, or one-fifth of the whole number of small squares (400). Therefore, to find the number of cells in the whole square m.m. the number counted is multiplied by five. The cubic contents of the counting chamber within the ruled square is 0.1 cubic m.m., therefore, multiply by ten to find the number in a whole cubic m.m. The blood has been diluted 200 times in filling the pipette, therefore, multiply by 200 to find the number of corpuscles in one cubic m.m. of undiluted blood.

Suppose the five blocks counted contained 96, 105, 98, 108 and 95 cells, the whole 80 squares then contain 502 cells; 502X5X10X200=5,020,000. The same result is obtained by the rule (to the sum of the corpuscles in 80 squares add four ciphers). It is customary to disregard figures below the hundred-thousand place, so the count in the above example is 5,000,000.

The normal number is given as 5,000,000, but higher

counts are often found, especially in young, vigorous adults, up to 6,000,000 not being unusual.

Counting Leucocytes.—

The leucocyte pipette has the mark 11 above the bulb. Acetic acid is used as diluting fluid, as it dissolves the erythrocytes and leaves only the leucocytes in the field. Diluting fluids, both for erythrocytes and leucocytes, should be filtered often enough to keep them perfectly clear.

Diluting fluid for leucocytes:

Glacial acetic acid, 1 c.c.
Distilled water, 99 c.c.

The pipette is filled and the drop mounted, as in counting reds. The low power is used for counting leucocytes, as with it a square millimeter, which is the unit in counting white cells can be got into the field. With a Türck counting slide the central square is ruled into 400 small squares, as described in counting red cells, and this square is surrounded by eight other squares of equal size but less closely ruled. These outside squares are better for counting leucocytes, as there are fewer lines to confuse the eye. A good rule is to count all the cells in each corner square, average the result and multiply by 200.

Example.—Suppose the square millimeters counted contained 45, 50, 47, 42. The average number is 46. The depth of the cell is 0.1 millimeter; therefore, to find the number in a whole cubic millimeter one must multiply by ten. The dilution is 20. 45X10X20=9,200, the number of cells per cubic millimeter of undiluted blood.

Cleansing the Haemocytometer.—

Use nothing on the slide but water. Dry slide and cover

glass with a soft cloth. Old handkerchiefs should be kept for this purpose.

Blow the fluid from the pipettes, then draw up and blow out, first, water, second, alcohol and, third, ether. Lastly, draw air through pipette until the glass ball rolls freely. A soft rubber bulb, such as is used for an ear syringe, with an inch cut off the tube, is useful for cleansing pipettes, both to draw up fluids and to blow out fluids and air.

Color Index.—

The term color index indicates the haemoglobin content of the erythrocyte, as compared with a normal standard. Five million is taken as the standard erythrocyte count and is called 100 per cent. The standard of haemoglobin is 100 per cent, as estimated by the ordinary instruments.

$$\text{Color index} = \frac{\%\text{ of haemoglobin.}}{\%\text{ of erythrocytes.}}$$

Example.—The haemoglobin is 80%.

The erythrocyte count is 5,000,000 (100%).

80÷100=0.8=color index.

To obtain the per cent of the red cell count, multiply the first two figures of the count by two.

Making and Staining Blood Films.—

Slides and cover glasses must be clean and well polished. Washing in water and drying with a soft cloth is usually sufficient.

Making Films: Touch a slide near its end to a small drop of blood. Place one end of a second slide against the surface of the first and draw it up to the drop of blood so that the blood is in the acute angle. Push the spreader slide slowly along the surface of the first slide. The blood will be drawn along into a thin film.

Staining Films: Most modern blood stains are made up with methyl alcohol, which fixes the film. As soon as the film is dried in the air it is ready for staining.

Wright's Blood Stain is the most satisfactory for general use. It is difficult to prepare and can be satisfactorily purchased. That obtained from E. H. Sargent & Company is reliable. The original technique for staining gives the best results.

1. Drop stain upon air dried film until the film is well covered. Allow to stand one minute.
2. Drop on distilled water until metallic lustre appears. It usually takes a little more water than stain. Allow to stand three or four minutes.
3. Wash in distilled water until film looks pink. Drain or dry with filter paper. Examine with oil immersion lens.

Effect of the Stain.

1. Erythrocytes stain pink or yellowish pink.
2. All nuclei stain blue or purplish; nuclei of lymphocytes deep purplish blue, of large mononuclear leucocytes lighter blue, of granular cells dark blue.
3. Cytoplasm of lymphocytes stains robin's egg blue, of large mononuclears transparent pale blue, of granular cells faintly pink or not at all.
4. Granules. Neutrophile granules stain violet, eosinophile granules bright red, basophile granules dark purplish blue.
5. Blood platelets stain blue or lilac.
6. Malarial parasites stain blue, with the chromatin body reddish purple.
7. Bacteria stain blue.

Leucocytes, varieties, percentage of each kind and appearance in the stained film.

1. *Small lymphocytes.* 15% to 30%. Smaller than granular leucocytes. (7 to 11 μ.) The nucleus is large, round, deeply staining and is surrounded by a relatively small amount of cytoplasm which takes the basic stain (light blue).

2. *Large mononuclear cells.* 3% to 10%. As large as or larger than granular cells.
   a. Large lymphocytes. Differ from small lymphocytes only in size or sometimes in staining less deeply. Not numerous in adult normal blood.
   b. Large mononuclear leucocytes. The nucleus is relatively small, single, round or oval, and is surrounded with a wide zone of faintly staining cytoplasm.
   c. Transitional cells. Differ from large mononuclear leucocytes only in having a deeply indented or horseshoe-shaped nucleus.

   a, b and c are usually classed together in making a differential leucocyte count.

3. *Polymorphonuclear neutrophiles.* 60% to 75%. Average size 11 μ. Irregular convoluted nucleus, often looking like separate nuclei. Cytoplasm filled with fine dust-like granules stained lilac color or dull pink.

4. *Eosinophiles.* 1% to 4%. Differ from neutrophiles only in having coarse round granules which stain bright red.

5. *Basophiles* or mast cells. 0.5% to 1%. Differ from neutrophiles in having large irregular-shaped granules which stain a deep purplish blue.

   3, 4 and 5 are the granular leucocytes. The granular leucocytes, the large mononuclear leucocytes and transitionals are formed in the bone marrow; the large and small leucocytes in lymphatic tissue.

The Differential Leucocyte Count.

The differential leucocyte count is made from the stained film, using the oil immersion lens. A list is made of the varieties of leucocytes, polymorphonuclear neutrophiles, small mononuclears, large mononuclears (including large lymphocytes, large mononuclear leucocytes and transitionals), eosinophiles, basophiles and myelocytes. The slide is examined systematically, so as to avoid counting the same area more than once, and each leucocyte seen is recorded under the proper heading. One hundred leucocytes are counted in this way. The number recorded under each heading is the percentage of that variety. Except when the first hundred is quite normal, the total leucocyte count being normal also, another hundred should be counted and the results averaged. When the differential count is of special importance, at least four hundreds should be counted.

Beginners usually set down a stroke for each cell counted, making four and crossing with the fifth. With practice a considerable number can be kept in the mind, so that it is necessary to record only a few times during the count, using figures instead of strokes.

Coagulation Time.

As a preliminary to surgical operations, as tonsillectomy or any surgical procedure in the presence of jaundice, it is often important to test the coagulability of the blood. The coagulation time is much increased in haemophilia, purpura and jaundice.

A modification of Rudolph's method is practical. A wide-mouthed bottle containing about one litre is fitted with a large cork, which is perforated for several glass tubes and a thermometer. The glass tubes may be made from tubing about one-fourth inch in diameter. They are long enough to reach nearly to the bottom of the bottle and extend a little above the cork, and are sealed at the lower end. The bottle is filled with

water, at the temperature of 20° C. Several capillary pipettes about one-sixteenth inch in diameter and as long as the tubes are each fitted with a rubber nipple. The ear or finger is punctured and the time of appearance of the drop noted. Enough blood is drawn into one of the capillary pipettes so that the column is two inches or more in length. It is then drawn high enough so that it is an inch or more from the end at which it entered. This end is sealed in the flame and the pipette inserted into one of the tubes. It is well to fill two or three pipettes in this manner. The blood of the person making the test may be used as a control if it is known to be normal.

At the end of four or five minutes from the time the puncture was made, draw out the first of the pipettes, score with a sharp file near the lower end of the column, break the pipette and separate the ends slowly. When the blood has coagulated the clot will draw out between the broken ends. If coagulation has not taken place other trials are made at intervals of a half minute until the clot is demonstrated. Normal blood coagulates in from five to eight minutes, as a rule. If the coagulation time is more than ten minutes it is distinctly retarded.

General Pathology of the Blood.

Erythrocytes may vary from the normal:

In number.—

1. Oligocythaemia is a decreased number of erythrocytes.

It occurs in:

a. All anaemias.

b. Blood dilution. This is a temporary condition after administration of salt solution or when transudates are being absorbed.

2. Polycythaemia is an increased number of erythrocytes. This is not a frequent finding.

It occurs in:

a. Loss of fluids, as after excessive diarrhoea, sweats or vomiting.
b. High altitudes, proportional to the height.
c. Phosphorus and CO poisoning.
d. Cyanosis, from any cause.
e. The new-born.
f. A few cases of very high counts of unknown origin.

In size.—

Macrocytes are erythrocytes above the normal size.
Microcytes are erythrocytes below the normal size.
The presence of macrocytes and microcytes is called anisocytosis.

In shape.—

The presence of red cells abnormal in shape is called poikilocytosis.

In staining properties.—

Polychromatophilia is a condition in which the red cells take other than the acid stain (eosin); that is, they stain more or less with the basic dye (methylene blue) and appear bluish or lead colored instead of pink. Basic stippling is a condition in which the basic stain shows in fine specks or dots.

In being nucleated.—

Normoblasts are nucleated reds of normal size.
Megaloblasts are nucleated reds of larger than normal size.
Microblasts are nucleated reds of smaller than normal size.

Megaloblasts are usually young cells with large, pale, reticulated nuclei and cytoplasm containing but little haemoglobin and therefore staining a bluish color. Normoblasts are usually older cells with smaller, deeply staining nuclei, and haemoglobin containing cytoplasm staining like non-nucleated reds. Microblasts are old, degenerated cells having often but little cytoplasm left and that taking the basic stain. Their nuclei stain very deeply. The presence of nucleated reds is abnormal in the circulating blood (except normoblasts in the new-born) and is evidence of abnormal stimulation of the blood forming marrow. The appearance of megaloblasts is of more serious significance than the appearance of normoblasts.

Leucocytes.—

Leucocytes vary from the normal in number, in proportion of the different varieties, and in the appearance of the myelocyte, normally found only in the bone marrow.

The myelocyte is a large mononuclear cell with granules. The nucleus is round, oval or slightly indented and often eccentrically placed. There are three varieties, named according to their granules, neutrophilic myelocytes, eosinophilic myelocytes and basophilic myelocytes. They stain less perfectly than normal leucocytes, both as to nuclei and granules, and most of them are larger (18 μ or 20 μ). These cells are the parent cells of the granular leucocytes. Myeloid leukemia is the only condition in which myelocytes appear in the circulating blood in large numbers. A few may be present in any severe anaemia or pronounced leucocytosis.

Leucocytosis is an increase in the number of leucocytes in the circulating blood. The term is commonly used to mean that kind of leucocyte increase which appears as a response to most infections, that is, a polynucleosis.

Leucocytosis (polynucleosis) occurs:

1. Physiologically.
   a. In the new-born, lasting a few days.
   b. After a full meal (not pronounced).
2. Pathologically.
   a. In most infections and febrile diseases, except typhoid, malaria, measles, influenza and uncomplicated tuberculosis.
   b. After hemorrhage.
   c. In malignant disease.
   d. From various toxic and medicinal causes.

Lymphocytosis is an increase in lymphocytes, either absolute (increase in total number) or relative (increase in percentage).

It occurs:

1. Physiologically, in childhood (relative).
2. Pathologically.
   a. In whooping cough (absolute as well as relative), may be high and may occur early enough to aid in diagnosis.
   b. In syphilis, especially hereditary (relative).
   c. In malaria (relative). The large mononuclear is increased.
   d. In leucopenia from any cause (relative), due to decrease of polynuclear cells, for example in typhoid and pernicious anaemia.
   e. In lymphatic leukemia. The highest total count and greatest proportion of lymphocytes occur in this disease.

Eosinophilia is an increase in eosinophiles in the circulating blood.

It occurs:

1. In bronchial asthma.
2. In skin eruptions from any cause.
3. When there are intestinal parasites, notably in trichinosis.
4. In myelogenous leukemia. Eosinophiles are increased with every form of granular cells.

Increase in Basophiles or Mast cells.—

1. In myeloid leukemia.
2. Whenever eosinophiles are increased, basophiles are apt to be somewhat increased.

Blood Diseases.

Anaemia is a deficiency in red cell substance, that is, in haemoglobin, in number of red cells or in both.

Secondary anaemia.—

Typical blood picture:—

Haemoglobin reduced.
R. B. C. reduced.
Color index low.
W. B. C. moderate leucocytosis.
Stained film: qualitative changes only in severe cases.
Normoblasts, rarely megaloblasts, may be present.

Chief causes, and special characteristics.

1. Acute hemorrhage, as from trauma, abortion, tubal pregnancy, typhoid, pulmonary tuberculosis.

anaemia appears quickly and recovers quickly, the count becoming normal sooner than the haemoglobin.

2. Chronic hemorrhages, as from hemorrhoids, fibroid tumors of the uterus, cancer of the stomach.
   Haemoglobin may be very low, the red cell count not so low, fewer blasts, recovery slow.

3. Blood poisons.
   a. Infectious diseases.
   b. Malignant tumors, the degree of anaemia in proportion to malignancy.
   c. Chemical poisons, e. g., lead, mercury, arsenic and the coal tar derivatives. In lead poisoning basic stippling is found early and blasts are numerous.
   d. Chronic wasting diseases, e. g., lues, nephritis, rickets.

4. Poor food, inanition and unhygienic surroundings.

5. Parasites, as hookworm, sometimes tapeworm. The anaemia may be severe and show qualitative changes in red cells.

6. Malaria. Anaemia results from direct destruction of red cells. There is a relative increase of the large mononuclear, but no increase in the total leucocyte count. There may be leucopenia. Late severe malarial cachexias show a blood picture like pernicious anaemia.

Chlorosis.—

Chlorosis is a disease of defective blood formation. There is no evidence of blood destruction. It is not diagnosed by the blood alone.

Blood picture:—

Haemoglobin low. This is the essential change. R. B. C. count moderately low, or may be normal. Color index very

low, often 0.5. W. B. C. count normal or slightly reduced. Stained film: All the red cells show lack of haemoglobin and are slightly undersized. Qualitative changes are absent or inconspicuous. Normoblasts may be found in severe cases. Blood plates are increased. There is a tendency to relative lymphocytosis.

Pernicious Anaemia.

The etiology is unknown. Haemolysis is the chief change. Whether the haemolytic agent acts in the blood-making marrow or in the circulation is not known. The only lesions constantly found post-mortem are hyperplasia of red bone marrow and increased iron pigment in the liver. The disease is nearly always finally fatal, but three or four remissions may occur, lasting from a few days to many months. The blood examination is necessary to a diagnosis, but clinical history and symptoms are also important.

Blood picture:—

Appearance, pale, watery and slow to coagulate.

Haemoglobin, low but not so reduced as the red cell count. R. B. C. count very low, the most striking change, (1,000,000 to 2,000,000 common, has been reported as low as 150,000).

Color index high, the only condition in which it is high. The higher the color index the worse the prognosis.

W. B. C. count low. Leucopenia may be pronounced (3,500 common, has been reported as low as 500).

Stained smear: Cells scattered in the field. Qualitative changes marked, poikilocytosis, anisocytosis (more macrocytes than microcytes), polychromatophilia and basic stippling all present in typical cases. Variation in haemoglobin content is shown by dark and pale staining cells. The

more macrocytes with excess of haemoglobin, the higher the color index. Nucleated reds are present in all cases, but vary in number at different times. Typically, megaloblasts exceed normoblasts. The differential leucocyte count shows a relative lymphocytosis proportional to the leucopenia. Eosinophiles are decreased when the disease is progressing and increased when there is improvement. An occasional myelocyte may be found. Blood plates are reduced in number.

Leukemia.—

Leukemia is a condition characterized by persistent increase in leucocytes, and changes in lymph glands, spleen and marrow.

There are two types of blood findings: 1st, the myeloid, in which there is an overproduction of those leucocytes formed in the marrow, that is, all kinds except lymphocytes; 2nd, the lymphatic, in which there is an overproduction of lymphocytes.

In the myeloid form there is hyperplasia of the myeloid marrow, and myeloid tissue may appear in the spleen, lymph glands, liver and many other places in the body. In the lymphatic form lymph tissue may infiltrate the various organs and tissues of the body. Some pathologists regard leukemias as malignant tumors of the marrow and of lymphatic tissue. The tumor cells, being cells belonging to the circulating blood, are carried to all parts of the body and form metastases.

Either form of the disease may be acute or chronic in its course. Remissions sometimes occur in which the leucocyte count may be normal, but examination of a stained smear usually shows the presence of abnormal cells and abnormal percentages of the different varieties of leucocytes.

Blood picture in myeloid leukemia. (Synonyms, myelogenous leukemia, splenomyelogenous leukemia, myeloblastoma.)

Haemoglobin is reduced as the disease progresses. Estimation by usual methods is difficult because the large number of leucocytes changes the color and consistency of the blood.

R. B. C. count is little reduced at first, more later.

Color index low.

W. B. C. count enormously increased (250,000 common, may be 1,000,000).

Stained film: The diagnosis is often made at a glance from the great number of granular leucocytes in the field The differential count shows many myelocytes (30% average). All kinds of leucocytes except lymphocytes are increased. The percentage of the different varieties varies greatly. Qualitative changes in the reds are usual. Blasts are constantly found and are more numerous than in any other blood disease.

Blood picture in lymphatic leukemia. (Synonyms, lymphaemia, lymphoblastoma.)

Haemoglobin low.

R. B. C. count low.

Color index low.

W. B. C. count very high (150,000 common, may be 1,000,000, but is often as low as 30,000). Some acute cases show very moderate counts.

Stained film: Although the erythrocyte count is often lower than in myeloid leukemia on account of hemorrhages which are frequent in this condition, the qualitative changes are not so marked and nucleated reds are much fewer. The differential count usually shows 90% or more of lymphocytes. The other varieties of leucocytes are not much changed.

Diagnosis of Malaria from the blood examination.—

The plasmodia of malaria are found in the circulating blood during and shortly before and after febrile paroxysms. Their presence during quiescent periods is not easy to demonstrate. Quinine, even one dose, causes most of the parasites to disappear from the peripheral blood.

Methods of Examination:—

1. Moist preparation. Take a small drop of blood on a slide, cover with cover glass, pressing upon the cover glass to make the layer of blood thin. Examine at once. The parasites appear as hyaline, variously shaped bodies in the red cells. Except in their earliest stages they contain dark, brownish pigment which has a dancing motion. The pigment is usually the first thing to attract the eye. The parasite itself, except in the full-grown asexual and sexual forms, has a slow amoeboid motion.

2. The stained film. This method is preferred when the patient is not convenient to the laboratory. It is less liable to error in the hands of the inexperienced.

With Wright's stain the parasite stains blue with a reddish chromatin body within it. The pigment is unchanged, looking about the same as in the unstained blood.

In *tertian* and *quartan* fevers the asexual forms are numerous in the peripheral blood and the occasionally found sexual forms are not easily distinguished from the full-grown asexual.

In *aestivo-autumnal* fever the asexual forms are at first seen in the peripheral blood, but their later development takes place in the spleen. The sexual form of this variety, the crescent, is distinctive and is constantly found in the peripheral blood after the first few days of the infection. These

crescents are resistant to quinine and often remain in the blood a long time.

The blood in malaria early in the infection may show nothing abnormal but the presence of the parasites. Anaemia develops rapidly and in long-standing cases the blood may show a picture much like that of pernicious anaemia.

CHAPTER II.

SERUM DIAGNOSIS.

Widal Reaction.

The Typhoid Culture.—

Old laboratory cultures which have been many times transplanted are often better, being more motile than freshly-isolated bacteria. For making the test use an 18 to 24-hour bouillon culture which has been inoculated from the stock agar culture and incubated. At this stage the bacilli are actively motile and are about numerous enough to make a good field. Examine a loopful of the culture under the high dry lens to see that the bacilli are motile and are not spontaneously clumped.

Obtaining the Blood or Serum.—

The blood is obtained from a puncture as for a blood count. Whole blood may be used. It can be diluted in a white blood counting pipette. The disadvantage is that the corpuscles obscure the field. Blood serum is the best form to use. Obtain the blood in a capillary tube (Wright capsule), centrifuge to separate the serum, or let stand until the serum separates. Dried blood may be used, will keep for some time and is convenient for sending to the laboratory. Large drops of blood are allowed to drop on glass slides or glazed paper and are dried in the air.

Dilution of the Serum.—

A dilution of 1 to 40 or higher is required that the test may have any diagnostic value, because normal blood undiluted or 1 to 20 will sometimes clump typhoid bacilli.

For whole blood.—Draw the blood to 1 mark in white blood counter, draw up physiological salt solution to mark 11 and mix. This makes 1 in 20 dilution (blood is half serum). Blow out into a watch glass.

For serum.—Remove the serum with capillary pipette. To one drop of serum add 19 drops of physiological salt solution. This makes 1 in 20 dilution.

For dried blood.—Scrape the dried blood from the slide into a watch crystal. Add 9 drops of salt solution. This makes dilution 1 in 20 (dried blood is half serum).

Making the Mixture.—

Place a loopful of the 1 in 20 dilution of serum on a cover glass. Mix with a loopful of the bouillon culture. The fluid of the culture doubles the dilution, making 1 in 40 dilution of the serum. Ring a hollow ground slide with vaseline and mount the mixture as a hanging drop.

Observing the Reaction.—

Use the high dry lens and subdued light. Artificial light is sometimes better than daylight. The field should show at first actively motile separate bacilli. If the reaction is positive, in from a few minutes to 1 hour the motion gradually slows and ceases, and the bacilli gather into groups. The reaction is more or less marked according to the amount of agglutinin present. To call a reaction positive the majority of the bacilli must have stopped their motion

and there must be many clumps of 5 or more bacilli. For a 1 in 40 dilution 40 minutes' time of observance is enough; for higher dilutions, 1 hour.

Value of Widal Reaction in Typhoid Fever.

In typhoid fever the Widal reaction rarely fails to appear, and is our most certain confirmatory test after the bacilli have left the blood. During the first week of the disease the typhoid bacilli may be found in the blood. The Widal appears first on the seventh or eighth day, usually continues well into convalescence, and may continue an indefinite time after recovery.

The Wassermann Test for Syphilis.

Haemolysis.—

If the erythrocytes of an animal, for example, a sheep, are injected into the blood stream of an animal of another species, for example, a rabbit, the blood of the animal receiving the corpuscles (the rabbit) gains power to dissolve the erythrocytes of the species from which the corpuscles come (sheep) that is, becomes immunized to these corpuscles. But if the serum of the rabbit is heated to 56° C. for one-half hour (inactivated), or kept at room temperature for twenty-four hours, it loses this power. Haemolysing power may be restored to this serum by adding to it some fresh unheated serum from any animal. The haemolysis depends upon two substances present in the serum of the immunized animal, one destroyed by heat (complement) and the other not so destroyed (amboceptor).

A haemolytic system consists of a suspension of erythrocytes, the serum of an animal immunized to these erythrocytes, and complement (contained in fresh unheated serum of any animal).

Complement Fixation.—

Complement is present in all sera and will react with different kinds of amboceptors, but amboceptors are developed as the result of the introduction into the blood of some foreign material; for example, erythrocytes of another species give rise to haemolytic amboceptors. Bacteria give rise to bacteriolytic amboceptors, which react in a similar manner with complement and the invading bacteria.

The substance giving rise to these amboceptors is called antigen; the corpuscles are the antigen in the haemolytic system, the bacteria are the antigen in the bacteriolytic system.

If a bacteriolytic antigen, the corresponding amboceptor and complement are incubated together until the combination has had time to take place, and then the antigen of a haemolytic system, that is, erythrocytes, and haemolytic amboceptor are added to the former combination, no haemolysis will take place, because the complement has been used up or bound in the first system. If, however, the first system contained no amboceptor, then haemolysis would take place, as the complement would be free to unite with the haemolytic system.

The Wassermann reaction in syphilis seeks to demonstrate, by this method, the presence or absence of syphilitic amboceptor in a patient's blood.

In general terms the proceeding in the Wassermann reaction is this: The patient's blood serum, inactivated to remove its own complement, is combined with complement supplied by fresh guinea pig serum, and syphilitic antigen. These three substances are incubated together until combination of complement, antigen and amboceptor has had time to take place if the patient's serum contains syphilitic amboceptor. There is no visible change to show whether

combination has taken place or not. If the patient's serum contains syphilitic amboceptor and this has combined with the antigen and complement, then there will be no free complement left in the mixture. Adding another system of antigen and amboceptor, one in which the combination, if it takes place, will cause a visible change, will then show whether there is complement in the mixture free to act in the latter system. A haemolytic system is used, as haemolysis is a plainly visible phenomenon.

After an hour's incubation haemolytic amboceptor (the serum of a rabbit immunized to sheep's erythrocytes) and washed sheep's corpuscles are added and the mixture again incubated. If there is free complement it will unite with the haemolytic amboceptor and sheep's corpuscles and dissolve the corpuscles. If the complement has been used in the former combination, the sheep's cells will not be dissolved. Absence of haemolysis, then, indicates that the patient's blood contains syphilitic amboceptor, that is, the reaction is positive. Haemolysis shows that the complement was not used in the first combination, owing to the absence of the third necessary element, syphilitic amboceptor in the patient's blood, but was free to enter into the haemolytic system and bring about haemolysis of the sheep's cells, that is, a negative reaction.

Preparation of Materials Used in the Test.—

(1) **Sheep Corpuscles.**—If the sheep's blood is obtained from a slaughter house it must be used within a day or two, as bacterial contamination will soon cause haemolysis. But if obtained in a sterile manner and kept sterile the corpuscles may be used for a week, as a rule. In either case, as soon as the blood is drawn it must be defibrinated by shaking five minutes in a bottle with bits of glass or wire. It is then kept in a refrigerator until time to prepare for use.

(2) **Haemolytic Amboceptor.**—The inactivated serum of a rabbit which has been immunized to sheep erythrocytes furnishes the haemolytic amboceptor. Two intravenous injections of one to two c.c. of washed sheep corpuscles are given to a rabbit at an interval of four or five days. Five to seven days after the second injection the rabbit's serum will contain the maximum amboceptor. At this time the rabbit is bled from the ear vein, the serum tested, and if found of sufficient strength to use in a dilution as high as 1 to 1,000, the rabbit is anaesthetized and bled from the carotid with aseptic precautions. The serum is separated by the centrifuge and inactivated at 56° C. for one-half hour, to destroy its complement. It is well to inject two or three rabbits at a time, as not every one injected produces good amboceptor. The amboceptor is stored in sealed glass tubes and kept in the refrigerator.

(3) **Complement.**—Guinea pig serum is used as its complement content is high and rather constant. The animal may be bled by puncturing the heart with a hypodermic needle and withdrawing four or five c.c. of blood into the syringe. This requires some skill and many prefer to kill the pig and bleed from the vessels of the neck. The blood should stand until coagulation has taken place. The serum is separated by the centrifuge and pipetted from the clot. It must be used within twenty-four hours unless it is kept frozen. The serum can be placed in small tubes with water-tight stoppers and buried in salt and ice in a thermos bottle. By renewing the salt and ice every day or two the complement will be kept frozen and will remain potent for weeks. Just what is needed for a set of tests may be melted at the time the tests are made.

(4) **Antigen.**—The substances used as antigens in the Wassermann reaction are not biologically specific antigens. Various lipoid substances have been found to bind comple-

ment in the presence of syphilitic serum and not to bind it in the presence of non-syphilitic serum.

Among the substances found most successful as antigens are, first, alcoholic extracts of syphilitic liver; second, alcoholic extracts of normal organs, as guinea pig hearts; third, cholesterinized extracts of normal organs, as guinea pig, beef or human heart muscle. These extracts are not always of the same strength nor enduring quality.

(5) **The Patient's Blood Serum.**—The blood is obtained from a vein at the bend of the elbow. A ten c.c. Luer syringe with a No. 19 needle is convenient for puncturing the vein and obtaining the blood, or the blood may be allowed to drop directly through the needle into a sterile tube. It is well to take as much as 5 c.c. of blood, although less may be sufficient. The blood is allowed to stand until coagulated. The serum is separated by the centrifuge and pipetted from the clot. The serum is inactivated in a water bath at 56° C. for one-half hour to destroy its complement. If the serum is not used within a few hours it should be kept in a refrigerator. If handled in a manner to prevent contamination the serum will be good for the test for a few days, but more reliable results are obtained if the test is made within forty-eight hours after the blood is taken.

The glassware should be clean and dry. All tubes and pipettes should be thoroughly washed in hot running water. A supply of test tubes ½ x 4 inches is required. This size allows the contents to be well mixed by shaking.

Racks to fit the tubes with holes arranged in parallel rows are useful. Five rows of a dozen in a row form a good rack. A water bath with the same arrangement for tubes is desirable. One c.c. outflow pipettes marked in tenths are used and a good supply of them is needed.

Sterile centrifuge tubes, and a good supply of capillary pipettes with rubber nipples, should be on hand.

0.85% sodium chloride solution is used for diluting all reagents.

Diluting and Titrating Reagents Ready for Test.

1. **Corpuscles.**—A few c.c. of the defibrinated sheep's blood are put into a centrifuge tube, the tube filled with 0.85% sodium chloride solution and centrifuged until the corpuscles are settled. The fluid is pipetted off, and the washing repeated twice, mixing the corpuscles well with the salt solution each time. After the last washing all the salt solution is carefully pipetted off. A 2.5% suspension of the sheep cells is made by taking 1 c.c. of the cells and 39 c.c. of salt solution; 0.5 c.c. of this suspension is used in the test.

2. **Amboceptor.**—The amboceptor may be diluted 1-1000 for the first trial. In order not to waste amboceptor it is well to dilute one drop with nine drops of salt solution, and make the higher dilutions from this. 0.1 c.c. of this 1-10 dilution added to 9.9 c.c. salt solution will make the 1-1000 dilution.

To titrate the amboceptor when the strength of the complement is still unknown it is necessary to use a probable excess of complement; 0.5 c.c. of a 1-10 dilution of complement will usually be enough.

A series of tubes is prepared, each containing 1 c.c. salt solution, 0.5 c.c. corpuscle suspension, and 0.5 c.c. complement dilution 1-10. To these add increasing doses of 1-1,000 dilution of amboceptor, to the first 0.1 c.c., to the second 0.2 c.c., and so on. Incubate one-half hour. The first tube which shows complete haemolysis contains one unit of amboceptor. Two units are used in the test.

3. **Complement.**—The amount of guinea pig serum needed for the set of tests is diluted 1-10 with salt solution. To titrate the complement a series of tubes is prepared,

each containing 1 c.c. salt solution, 0.5 c.c. corpuscle suspension and one unit of amboceptor. To these are added increasing amounts of complement, to the first 0.1 c.c., to the second 0.2 c.c., and so on. The tubes are incubated one-half hour. The first tube which shows complete haemolysis contains one unit of complement. One and one-half or two units are used in the test.

4. **Antigen.**—Antigen must be titrated to obtain its anticomplementary unit and also its antigenic unit. When these units are once obtained they usually remain the same for some time, and these titrations need not be repeated every time the test is made.

The anticomplementary unit.—

Antigens alone absorb some complement, and if used in large enough amount would prevent haemolysis in a haemolytic system without the presence of any serum or in the presence of a normal serum. The amount of antigen which will absorb or bind the two units of complement used in the test is the anticomplementary unit. To ascertain this a series of tubes is prepared, each containing 1 c.c. of salt solution, two units of complement, 0.1 c.c. of known normal serum. To these increasing amounts of antigen are added. The first tube may have 0.1 c.c. of a 1-10 dilution of antigen, the second 0.3 c.c., etc. These are incubated one-half hour in a water bath, then to each is added two units of amboceptor and 0.5 c.c. of corpuscle suspension. Incubate again for one-half hour. The first tube which shows imperfect haemolysis contains the anticomplementary unit of antigen. Not more than one-fourth of this amount should be used in the test.

The antigenic unit.—

This is the amount of antigen necessary to inhibit haemolysis with a syphilitic serum. To obtain this unit a series of tubes is prepared, each containing 1 c.c. salt solution, two

units of complement and 0.1 c.c. of known syphilitic serum. To these are added decreasing amounts of antigen, beginning with ¼ the anticomplementary unit. These are incubated one-half hour in a water bath and then to each tube two units of amboceptor and 0.5 c.c. of corpuscle suspension are added. The tubes are again incubated for one hour. The first tube which shows inhibition of haemolysis contains the antigenic unit. This should be much less than one-fourth the anticomplementary unit in a good antigen. An abundance of antigen should be used in the test. One-fourth the anticomplementary unit is often used rather than smaller amounts. A fresh dilution of the antigen is prepared for each set of tests, according to the strength of the antigen. It is convenient to dilute the antigen enough so that the amount used in the test is contained in 0.5 c.c. of the dilution.

The Patient's and Control Sera. 0.1 c.c. of inactivated serum is used in the tests.

Making the Test.

These titrated and diluted reagents and the inactivated sera are arranged in convenient order. Tubes ½x4 inches are numbered in blue pencil as indicated in the chart. The chart indicates the order of the tubes in the rack and the contents of each tube. The rows are lengthened for other sera to be tested and other controls. More rows are added for other antigens, if more than one is used. At the end of two hours the reaction is usually complete, but it is well to place the tubes in the refrigerator and make a final reading next morning. The front row of tubes, 1, 3 and 5, containing no antigen should show complete haemolysis. The second row, 2, 4 and 6, will show no haemolysis in 2, haemolysis in 4, and haemolysis in 6 if the unknown serum is negative and none or little of it is positive.

DIAGRAM OF THE WASSERMANN TEST.

Tube 1	Tube 3	Tube 5
Positive serum 0.1 c.c. Complement 2 units. Salt solution 1 c.c. (Incubate 1 hour and add) Amboceptor 2 units Corpuscle suspension 0.5 c.c.	Negative serum 0.1 c.c. Complement 2 units. Salt solution 1 c.c. (Incubate 1 hour and add) Amboceptor 2 units Corpuscle suspension 0.5 c.c.	Unknown serum 0.1 c.c. Complement 2 units. Salt solution 1 c.c. (Incubate 1 hour and add) Amboceptor 2 units Corpuscle suspension 0.5 c.c.
Tube 2	**Tube 4**	**Tube 6**
Positive serum 0.1 c.c. Complement 2 units. Antigen dilution *0.5 c.c. Salt solution 1 c.c. (Incubate 1 hour and add**) Amboceptor 2 units Corpuscle suspension 0.5 c.c.	Negative serum 0.1 c.c. Complement 2 units. Antigen dilution 0.5 c.c. Salt solution 1 c.c. (Incubate 1 hour and add) Amboceptor 2 units Corpuscle suspension 0.5 c.c.	Unknown serum 0.1 c.c. Complement 2 units. Antigen dilution 0.5 c.c. Salt solution 1 c.c. (Incubate 1 hour and add) Amboceptor 2 units Corpuscle suspension 0.5 c.c.

After the addition of amboceptor and corpuscles the tubes are incubated two hours, and may then be set aside in the refrigerator for a final reading next morning.

*The antigen is diluted so that 0.5 c.c. contains the amount used in the test.

**If the incubation is in a water bath at 40°C., one-half hour is sufficient.

The test as described provides for the use of one antigen, but it is desirable to use two or more as in this way the tests are repeated. Sera which contain small amounts of antibody may give a clearer positive with one antigen than with another.

The antibody in a positive serum may be titrated by using decreasing amounts of the serum. A strongly positive serum will give a positive reaction with a fraction of the standard amount used in the test.

By substituting drops for tenths c.c. throughout, the Wassermann reaction may be made with smaller amounts of material. While this method is less accurate, it can be used with good results and when the serum is too small in amount for the c.c. method, it proves very convenient.

The Wassermann Test on spinal fluid is made just as the test on blood serum is made except that the spinal fluid is not inactivated, and that five to ten times the amount is used, that is 0.5 c.c. to 1 c.c. of undiluted fluid.

Diagnostic Value of the Wassermann Test.

A positive Wassermann may be obtained as early as four or five weeks after infection with syphilis, that is, a few days after the appearance of the chancre, but as a rule the reaction first becomes positive seven or eight weeks after infection and remains so throughout all stages of the disease if the case is untreated. In hereditary syphilis the reaction is positive in an equally high percentage of cases. There are occasional exceptions to this rule, a negative Wassermann being found in undoubted cases of active syphilis. Treatment renders the reaction negative in many cases, but a positive reaction recurs if the treatment has been insufficient to cure the disease. Three weeks should elapse after the cessation of treatment before the test is made. In syphilitic diseases of the nervous system the blood Wassermann may be negative and the spinal fluid Wassermann positive.

Positive Wassermann reactions have been found in some cases of jaundice, leprosy, relapsing fever, frambesia, trypanosomiasis and malaria in the febrile stage. The ingestion of alcohol may change a positive reaction to a negative.

Complement Fixation Test for Gonorrhoea.

The complement fixation test for gonorrhoea is performed in the same manner as the Wassermann test for syphilis, substituting gonococcus antigen for syphilitic antigen. Gonococcus antigen made from many strains of gonococci can be purchased from Parke, Davis & Co.

The diagnostic value of the complement fixation test for gonorrhoea is considerable, a positive reaction being more valuable than a negative. The test is seldom positive during the first few weeks of the infection but later in the course of the disease a large percentage of the cases show positive reactions.

CHAPTER III.

URINE.

Obtaining Specimens.

A twenty-four hour specimen is usually desirable, because the total output of both solids and water is learned in this way. A single specimen is sometimes useful, as sugar or albumin may be present in the urine at one time of the day, and not at other times.

Under ordinary circumstances no preservative is needed. When the urine must be transported a long distance or kept more than a day or two a preservative may be required. Formalin, one drop to four ounces of urine, will prevent decomposition. If more is added the formalin may reduce copper solutions used in sugar tests.

Physical Properties.

I. Amount. The normal quantity for an adult is from 1000 to 1500 c.c. in twenty-four hours. Children pass more in proportion to body weight than adults. A new born infant passes 150 to 200 c.c. A child of 5 years about 700 c.c.

In health more is passed during the day than during the night. This may be reversed in disease, notably in chronic interstitial nephritis.

The amount varies physiologically according to the

amount of fluids and watery foods taken and the activity of other organs of elimination.

Pathological factors influencing the amount are:

1. The condition of the renal parenchyma. For example there is oliguria or temporary anuria in acute nephritis.

2. The circulation in the kidney. The amount of urine is influenced by the rapidity of the blood flow, as well as by blood pressure. For example, there is oliguria in chronic passive congestion. Weak heart from any cause produces oliguria. Most diuretics increase the amount of urine by improving the circulation.

3. Abnormal quantity or quality of substances excreted. The sugar in diabetes causes polyuria. At the beginning of convalescence from acute fevers, accumulated waste products stimulate the kidney to increased output of urine.

4. Nervous causes. In hysteria there may be anuria, oliguria or polyuria. In various other nervous diseases variations in amount occur. The cause is thought to be vasomotor.

5. The unknown cause of diabetes insipidus produces polyuria.

II. Appearance.

Normal urine is transparent, though a slight cloud of mucus may appear on standing. Cloudiness is generally due to pus, blood, bacteria, urates or phosphates. Albumin itself even in large amounts causes no change in the appearance of the urine. The consistency of normal urine is watery, pathologically it may be frothy from the presence of albumin, syrupy from the presence of sugar, or ropy from mucus or pus in alkaline urine.

III. Color.

Normal urine is amber or straw colored. It may vary a great deal within normal limits.

Pathological Changes of Color:

1. Blue or green. The administration of methylene blue usually accounts for a blue or greenish color. Rarely a bluish urine appears from putrefactive changes in the intestines in cholera.

2. Dark yellow or greenish brown color is usually due to bile

3. Black. Melanin from a melanotic sarcoma may color the urine black. In salol, carbolic acid or iodoform poisoning, the urine may turn black on standing.

4. Red, brownish or smoky color may be due to blood.

5. Red color in alkaline urine may be due to phenolphthalein, rhubarb, senna or cascara.

6. Milky color is found when there is an admixture of chyle.

IV. Reaction.

Normal urine is usually acid in reaction but may be temporarily neutral or alkaline. An amphoteric reaction, the urine turning red litmus paper blue, and blue paper red is not unusual, and has no pathological significance. Alkaline urine may be due to decomposition in the bladder as in cystitis with retention of the urine, or to admistration of alkaline drugs. A meat diet has the tendency to increase the acidity, and fruit and vegetable diet to diminish acidity.

The reaction is determined by litmus paper, acid urine turning blue litmus red, and alkaline urine turning red litmus blue.

Total Acidity.—

The total acidity of urine varies considerably normally. The average normal acidity is 400° to 600°. In estimating the total acidity the specimen of urine should be a mixture of the twenty-four hour output and should be as fresh as possible.

Folin's Method of Estimation.—

Measure 10 c.c. of urine into a 100 c.c. flask, add about 25 c.c. of distilled water, 10 drops of 1% alcoholic solution of phenolphthalein and about 2 grams of potassium oxalate. Add slowly N/10 NaOH until a permanent faint pink color appears. The flask should be shaken after each addition of alkali and the alkali added drop by drop after the first one or two c.c. has been allowed to run in. The number of c.c. of N/10 NaOH required to neutralize 1 c.c. of urine multiplied by the number of c.c. of urine passed in 24 hours gives the degree of total acidity.

V. Specific Gravity.

The normal specific gravity of a 24 hour specimen of urine is between 1.012 and 1.020. Normally a great variation occurs in separate passages of urine, the night urine having a high specific gravity.

Physiologically the specific gravity varies inversely as the quantity. It is increased by nitrogenous diet. Pathologically the specific gravity is high in the following diseases:

1. Diabetes mellitus.
2. Acute nephritis.
3. Acute fevers.

The specific gravity is low in:

1. Diabetes insipidus.

2. Chronic interstitial nephritis. The specific gravity is markedly fixed and low, due to inability of the kidney to excrete a concentrated urine.

The specific gravity is estimated by means of the Squibbs urinometer. Dr. W. S. Harpole's Practical Urinometer enables one to take the specific gravity of a very small amount of urine.

Total Solids.—

By the total solids is meant the solids excreted in 24 hours. The chief value of the estimation of the specific gravity is to give an idea of the amount of solids excreted. The normal total solids of an adult on ordinary diet is about 60 grams. About 4% of the weight of the urine is that of the solids and of this urea forms a little less than half.

The total solids are estimated by the use of Haeser's coefficient. The last two figures of the specific gravity multiplied by 2.33 (Haeser's coefficient) equals the grams per litre. This multiplied by the number of litres passed in 24 hours gives the total solids. Example: 1400 c.c. of urine is the 24 hour amount. The specific gravity is 1.016. 16x2.33x1.4=52.19. The total solids are 52.19 grams.

Chemical Composition of Normal Urine.

The 24 hour output consists of:

1. Water, 1000 to 1500 c.c.
2. Solids, 60 grams.
    a. urea, 30 grams.
    b. chlorides, 15 grams.
    c. sulphates, 2.5 grams.
    d. phosphates, 2.5 grams.

e. ammonia, 0.7 gram.
f. uric acid, 0.7 gram.
g. traces of numerous other substances.

The amount of water and the various solids varies within wide limits in health, the above figures only serving to indicate the average output.

Abnormal Substances Found in Solution in Urine.

1. Proteids. Serum albumin, serum globulin, albumoses, nucleo-albumin, Bence-Jones proteid, mucin.

2. Carbo-hydrates. Glucose, lactose, rarely other sugars.

3. Acetone bodies. Acetone, diacetic acid and oxy-butyric acid.

4. Bile.

5. Indican.

Albuminuria.

True albuminuria is the presence in the urine of albumin which has escaped through the cortex of the kidney. The conditions in which true albuminuria occurs are classified as follows:

I. Albuminuria with definite renal lesions (usually large amounts of albumin).

1. Nephritis in all its forms.
2. Chronic passive congestion.
3. Acute congestion of the kidney.

II. Albuminuria without definite renal lesions (usually small amounts of albumin).

1. Functional. After severe exercise, after prolonged cold baths, in the new born, cylic albuminuria.

2. Febrile.
3. Haematogenous. In severe anaemias and cachexias, and in chronic diseases as lues.
4. Nervous. Usually transitory.

False or accidental albuminuria is the presence in the urine of albumin from the admixture of pus or blood outside the kidney substance, as in cystitis or injuries to the ureter, bladder or urethra.

Serum albumin and serum globulin are usually both present in albuminuria, sometimes also nucleo-albumin and albumoses. Most ordinary tests respond to all these substances. The appearance of proteoses or globulin except in association with serum albumin is rare. The Bence-Jones proteid appears in the urine in multiple myeloma. When present there is usually a large amount. This proteid coagulates on heating at about 60° C., clears up on further heating and reappears on cooling.

For all albumin tests the urine must be clear. If cloudy, filter. If still cloudy, mix the urine with light magnesia (magnesium oxide) and filter.

Qualitative Tests for Albumin.—

Heller's Test. Take an inch of urine in a test tube, underlay this with half an inch of nitric acid. If the urine is alkaline, acidify it with a few drops of dilute acetic acid before making the test.

Interpretation of the test:

Precipitates.

1. Albumin shows a white ring at junction of acid and urine.
2. Nucleo albumin or mucin shows white ring above the contact with clear zone between.

3. Urates show a cloud above the contact line which disappears on heating or on using diluted urine.

4. Oleo-resins show a milkiness at the contact. They are not coagulated by heat.

Colors.

1. Normal—varies from faint pink to brownish ring at contact.
2. Biliary pigment—dark green or blue.
3. Indican—violet or bluish when great excess is present
4. Iodides—intense brownish red.
5. Anilin compounds—red to purple.

Robert's Modification of Heller's Test. Use in place of nitric acid a reagent consisting of saturated aqueous solution of magnesium sulphate 100 c.c. and 20 c.c. of nitric acid.

The interpretation is the same as that of Heller's test so far as the precipitates are concerned and the test is more delicate.

Purdy's Test for Serum Albumin.—

Take 1/2 test tube full of clear urine, add 1/6 its volume of saturated solution of sodium chloride and 5 drops of 50% acetic acid. Boil the upper portion. A cloud in the boiled portion indicates serum albumin.

Quantitative Tests for Albumin.

Purdy's Centrifuge Method.—

Place 10 c.c. of urine in a graduated centrifuge tube. Add 3 c.c. of a 10% solution of potassium ferrocyanide, and 2 c.c. of 50% acetic acid. Mix by turning, let stand 5 minutes, then centrifuge three minutes at 1500 revolutions per minute. Read the percentage of the precipitate. Each mark represents 1%. The percentage by weight is approximately 1/50 of the bulk percentage as estimated by this method.

Tsuchiya's modification of the Esbach method.—

Use Esbach's albuminometer. Fill the tube to the mark U with urine. Add the reagent to the mark R. Mix by turning and let stand 24 hours. The scale reads in grams of albumin per litre of urine. To find the percentage move the decimal point one place to the left. If the precipitate comes up to the mark 4 the percentage is 0.4.

Tsuchiya's Reagent:

Phosphotungstic acid, 1.5 grams.
Concentrated HCl, 5 c.c.
95% alcohol q.s. 100 c.c.

Glycosuria.

Glycosuria is the presence of glucose in the urine. The assimilation limit for glucose is the minimum amount of sugar the ingestion of which is followed by sugar in the urine. The assimilation limit may be tested by giving 100 grams of glucose dissolved in ½ pint of water two hours after a breakfast of bread and coffee. Two hours later test the urine. If sugar is present the assimilation limit is pathologically lowered.

The assimilation limit is lowered in the following conditions: diabetes mellitus, pregnancy, starvation, acute diseases, exophthalmic goitre and destructive lesions of the liver and pancreas.

Spontaneous permanent glycosuria is nearly always due to diabetes mellitus.

Lactose is the only other sugar often found in the urine. This may appear when the function of lactation is interrupted, sometimes during lactation.

Qualitative Test for Glucose.—

Haines' Test.—

Boil an inch of Haines' solution in a test tube, add 5 drops of urine, bring to a boil; if no reaction occurs add 5 more drops of urine and bring to a boil again. If sugar is present a yellow or reddish yellow precipitate is thrown down. Avoid doubtful results by not using too much urine nor boiling too long. A slight greenish precipitate often appears on standing in urine containing no sugar.

Haines' Solution:
Cupric sulphate, 12 grams.
Potassium hydroxide, 45 grams.
Glycerine, 90 c.c.
Water q.s. 1000 c.c.

Quantitative Test for Glucose.

Haines' Method.—

Measure 10 c.c. of Haines' quantitative sugar test solution into a 100 c.c. flask and add 50 c.c. of water. Dilute the urine by adding 4 parts of water to 1 part of urine and fill graduated burette with diluted urine. Bring the Haines' solution in the flask to a boil. Allow the diluted urine to run from the burette into the boiling solution very slowly and finally drop by drop until the blue color disappears. 10 c.c. of Haines' solution is decolorized by 0.01 gram of glucose, therefore, whatever amount of urine was used to decolorize the Haines' solution in the flask contained 0.01 g. of glucose. 0.01 divided by the number of c.c. of undiluted urine used would give the grams of sugar in each c.c. of urine. 100 times this would give the amount in 100 c.c. or the per cent of sugar.

Example: 1 c.c. of urine decolorized the fluid in the flask. $0.01 \div 1 \times 100 = 1 = \%$ of sugar. If the 24 hour amount

of urine was 3000 c.c. the grams of sugar excreted in that time would be 1% of 3000 or 30 grams.

Haines' Solution for Quantitative Sugar Determination:

Copper sulphate.....8.314 grams
Potassium hydroxide.....25 grams
Ammonia .....350 c.c.
Glycerine .....40 c.c.
Distilled water q.s.....1000 c.c.

The Acetone Bodies.

B-Oxybutyric acid, diacetic acid and acetone are spoken of as the acetone bodies. One or more of them will appear in the urine when they are in excess in the blood. Poisoning by these bodies is called acidosis. This acidosis occurs when there is carbohydrate starvation. The source of the acetone bodies is the fats. The ammonia output in the urine is increased in proportion to the output of acetone bodies. The system is protected against the increased acidity due to the acetone bodies by neutralization with ammonia.

This sort of acid poisoning may occur, 1st, when carbohydrates are not ingested, as in starvation; 2nd, when carbohydrates cannot be retained or digested, as in cancer of the stomach and some intestinal disorders; 3rd, when carbohydrates cannot be assimilated, as in diabetes mellitus.

Test for acetone. (Gunning's)—

To ½ test tube of urine add about 1 c.c. each of tincture of iodine and ammonia. A black precipitate of nitrogen iodide forms and gradually disappears. If acetone is present iodoform is formed, and is recognized by its odor, yellow color and the microscopic appearance of its crystals.

Test for diacetic acid. (Gerhardt's)—

To ½ test tube of urine add a few drops of tincture of ferric chloride. If a precipitate (phosphates) occurs filter and add more ferric chloride to the filtrate. A dark red color which fades somewhat on boiling or on standing several hours, indicates diacetic acid. Salicylates, phenol and antipyrine cause a dark red color which does not fade on boiling.

Urea.

Normally about 85% of the nitrogen excreted in the urine is in the form of urea. This proportion is altered in acidosis because the nitrogen is taken up in forming the ammonia which combines with the acid.

Urea is increased when there is,

1. Increased intake of nitrogen, as with meat diet.
2. Increased tissue destruction, as in fevers or after excessive exercise.

Urea is decreased when there is,

1. Decreased intake of nitrogen in the food.
2. Destructive disease of the liver, as acute yellow atrophy.
3. Renal insufficiency as in acute nephritis and chronic interstitial nephritis.

Test for Urea.—

Use Doremus' ureometer. Remove albumin from urine if more than a trace is present, by acidifying with acetic acid, boiling and filtering. Fill the long arm of the ureometer with freshly made sodium hypobromite. Add slowly through the curved pipette exactly 1 c.c. of the urine. The gas evolved is nitrogen and bears a constant relation to

the amount of urea present. The percentage of urea is read on the scale.

Sodium hypobromite is made by adding 1 c.c. of bromine to 30 c.c. of a 20% solution of sodium hydroxide. The Doremus ureometer is filled with the 20% sodium hydroxide and the bromine introduced with a curved 1 c.c. pipette.

Ammonia.

The normal excretion of ammonia in the urine is less than one gram in twenty-four hours.

Notable increase in ammonia occurs in acidosis, the acid bodies being combined with ammonia to protect the tissues against the acids. In pernicious vomiting of pregnancy ammonia is much increased. The estimation of it may help to distinguish between pernicious vomiting and nervous vomiting. When the urea forming function of the liver is interfered with more of the nitrogenous waste appears as ammonia and correspondingly less as urea.

Formalin Method of Estimation.

To 10 c.c. of urine in a 100 c.c. flask add 25 c.c. of distilled water and 5 drops of a 1% alcoholic solution of phenolphthalein. Neutralize by the addition of N/10 NaOH. In a second flask measure 5 c.c. of formalin, add 25 c.c. of water and neutralize with N/10 NaOH, using phenolphthalein as an indicator. Add the neutralized formalin to the above neutralized urine. The resulting mixture will become colorless because the formalin breaks up the ammonium salts and liberates the acids. Titrate this acid in the mixture with N/10 NaOH. The number of c.c. N/10 NaOH used in this last titration X0.0017 will give the number of grams of NH^3 in 10 c.c. of urine. From this the amount in 24 hours can be calculated.

Example: Suppose 4 c.c. N/10 NaOH was used to

neutralize the acidity after adding the formalin. 4X0.0017=0.0068 grams of ammonia in 10 c.c. of urine. If 1000 c.c. is the amount of urine passed in 24 hours 0.0068X100=0.68 grams ammonia in 24 hours.

Uric Acid.

The source of the uric acid is the nucleins. It is present normally as urates of sodium, potassium and ammonium. It crystallizes in the urine when in excess, or when the urine is very concentrated and acid. The variations in amount are considerable in health.

Uric acid is increased:

1. After ingestion of foods rich in nucleins, as sweetbreads or liver.
2. In leukaemia, from the breaking down of many nucleated cells.
3. During and after the paroxysm in gout.
4. In fevers, corresponding to the increase in urea.

Uric acid is decreased:

1. In advanced kidney disease.
2. Preceding the paroxysm in gout.

Test for uric acid.—

Use Ruhemann's uricometer. Introduce through a pipette carbon disulphid up to the mark S. Add the iodine mixture to the mark J. Mix well. The carbon disulphid becomes a deep red color. Add urine up to the lowest calibration, mix by turning. Add more urine, a few drops at a time, turning after each addition, until the carbon disulphid is white. The reaction is then complete. Read the mark on the scale even with the surface of the urine. The scale reads in grams of uric acid per litre of urine.

Ruhemann's reagent:

Iodine, 0.5 g.
Potassium iodide, 1.25 g.
Absolute alcohol, 7.5 c.c.
Glycerine, 5 c.c.
Distilled water q.s., 100 c.c.

Indican.

Indol is a product of decomposition of proteid in the intestine. It is oxidized in the blood to indoxyl, combines with sulphuric acid to form indoxyl sulphate of potassium (indican), in which form it is excreted in the urine. An excess appears in

1. Intestinal putrefaction,
   a. Due to intestinal indigestion, diarrhoea, intestinal tuberculosis, constipation, etc.
   b. When HCl is lacking in the stomach as in chronic gastritis or cancer.
   c. When there is a lack of peristalsis as in peritonitis or ileus.

2. Putrefactive processes elsewhere in the body, as empyema, lung abscesses, advanced pulmonary tuberculosis.

Obermayer's Test.—

Pour ½ inch of urine into a test tube, add an equal amount of Obermayer's reagent and about 1 c.c. of chloroform. Mix well. If an excess of indican is present the chloroform will be colored blue, the depth of the color depending upon the amount of indican present.

Obermayer's Reagent:

Hydrochloric acid, 500 c.c.
Ferric chloride, 1 gram.

The HCl breaks up the potassium indoxyl sulphate and frees indoxyl. The ferric chloride oxidizes the indoxyl to indigo, which colors the chloroform blue.

In urine containing bile or in any highly colored urine a doubtful color may appear in this test. This is avoided by removing disturbing substances with lead acetate. To half a test tube of urine add 1/5 its volume of a saturate solution of lead acetate. Let stand a few minutes, filter and test filtrate.

Other oxidizing agents can be used with HCl in detecting indican.

The following test is more delicate than Obermayer's and shows a faint color with normal urine:

Pour 5 c.c. of HCl into a test tube, add 1 drop of HNO^3 and 15 drops of urine. Mix well. If indican is present an amethyst color appears, reaches its maximum in from 5 to 10 minutes and then changes to yellow.

Bile.

Bile in the urine is always pathological. It appears whenever bile is present in the blood, that is, in jaundice from any cause. Bile may appear in the urine before the jaundice is apparent in the skin. Bile salts and bile pigments usually appear together and tests for bile pigments usually suffice for both.

Tests.—

Urine containing bile has a very yellow or greenish color, it foams when shaken and the foam is distinctly yellow. It stains filter paper yellow. The formed elements in the sediment are stained yellow.

Gmelin's Test.—

Layer the urine with nitric acid. If bile is present a dark green color will be seen at the contact.

Smith-Rosen Test.—

Layer 2 c.c. of Smith's reagent upon an inch of urine. Emerald green ring appearing at the contact indicates bile.

Smith's reagent:

Tincture of iodine, 10 c.c.
95% alcohol, 90 c.c.

The Diazo Reaction of Ehrlich.

This reaction never occurs in health, and rarely in non-febrile diseases.

It is nearly always present in typhoid fever and measles, occasionally in pneumonia, scarlet fever, diphtheria, erysipelas and tuberculosis.

This reaction occurs in about 80% of the cases of typhoid, appearing early, often during the first week, and disappearing with the subsidence of the fever. Its reappearance during a relapse may serve to distinguish relapse from complication. The reaction is more constant and more marked in severe cases.

A positive Diazo assists in distinguishing measles from rötheln. The appearance of a positive Diazo in tuberculosis indicates a bad prognosis.

Test:

To 40 parts of solution I and one part of solution II (two fingers of solution 1+4 drops of solution 2) in a test tube add an equal amount of urine. Add quickly an excess of ammonia and shake. A deep red color and a pink foam constitute a positive reaction. A dark greenish precipitate appears within 24 hours.

Solution I:
Sulphanilic acid, 1 gram.
Hydrochloric acid, 5 c.c.
Distilled water, 100 c.c.
Solution II:
Sodium nitrite, 0.5 grams.
Distilled water, 100 c.c.
The solution of sodium nitrite should be freshly made.

Sediments in Urine.

Sediment in urine is never strictly normal but may not be of serious significance.

Methods of examination. A few facts may be obtained by macroscopic examination. Urates and phosphates may be recognized by appearance and chemical reactions. Uric acid in large amount is visible to the naked eye and characteristic in appearance. For microscopic examination the urine is centrifuged, the liquid poured off, the last drop in the centrifuge tube is shaken, then poured upon a slide, covered with a cover glass, and examined first with low power, afterwards with high, if it contains small bodies not easily identified with the low power. The light should be considerably cut down with the iris diaphragm as hyaline casts and other transparent bodies will be entirely invisible if there is too much light.

Unorganized or Chemical Sediments. Those which have no cellular form and no connection with the cellular elements of the body.

1. In acid urine.
   A. Amorphous.
      Urates, a heavy cloud usually pinkish or brick dust color, soluble on warming.
   B. Crystalline.
      a. *Uric acid.* Amber, rarely colorless, large crystals of various shapes, rhombic plates, diamonds, "whetstones" and "butcher's block" or short

cylinders. They tend to form in groups and masses or rosettes. They are soluble in caustic soda and insoluble in hydrochloric or acetic acid. They appear when uric acid is in excess and the urine is very acid and concentrated. They are only significant when found in a freshly voided specimen. They may form renal or vesical calculi, and may be found in the urine together with blood cells in this case.

b. *Oxalate of calcium.* Small, colorless, refractile. Common form is the envelope. Sheaf, hour glass and oval forms may appear. They vary much in size. They dissolve in strong hydrochloric acid. They appear after a diet rich in oxalates, as strawberries, rhubarb, tomatoes or spinach. They often accompany intestinal indigestion and muscular pains. They may form calculi and masses of them with blood cells may be found in this case.

c. *Cystin.* Colorless, six sided plates. They are rare. They are due to abnormal proteid metabolism and individuals sometimes have them in the urine throughout life. They form calculi.

d. *Leucin* and *tyrosin.* They appear together, the leucin in yellowish balls, the tyrosin as needle crystals in sheaves They are rare, being found in acute yellow atrophy of the liver and phosphorous poisoning.

2. In alkaline urine.

A. Amorphous.

a. *Phosphates.* Granular whitish cloud. Dissolves on the addition of acids.

b. *Carbonates.* Indistinguishable from phosphates in appearance. Dissolves on addition of acids

with effervescence. They indicate decomposition of the urine.

B. Crystalline.

a. *Ammonium-magnesium phosphate* (triple phosphate). Colorless, usually large, prism forms. The "coffin lid," "boot jack" and occasionally feathery forms are seen. They are soluble in acetic acid. They usually signify nothing but decomposing urine, but when found in fresh specimens they point to alkaline fermentation in the bladder.

b. *Ammonium urate.* The only urate which appears in alkaline urine. Brown prickly balls, "thorn apple" crystals, often grouped. They are soluble in acetic acid with formation of uric acid crystals. They appear only in decomposed urine containing free ammonia and have no pathological significance.

c. *Calcium carbonate.* Small indefinite "dumb bells" associated with amorphous phosphates.

Organized or Anatomical Sediments.—

1. *Epithelial cells.* Some are always present. The portion of the urinary tract from which the cells come cannot be definitely located. They are of three general types.

a. Squamous. Large, thin, flat, leaf like cells, often in sheets. Rather small round nucleus. From bladder, urethra or vagina.

b. Irregular cells. Caudate, pyramidal, cylindrical. May come from deep layers of epithelium anywhere in the tract.

c. Round cells. Small round cells with comparatively large nuclei, often slightly granular. These include the renal cells, but cells similar in appearance may come from other portions of the tract. Such cells are not common in normal urine.

In nephritis they are often seen to be in a state of fatty degeneration.

2. *Red blood cells.* Small round, homogeneous, non-nucleated cells. They may retain their normal disc shape and pale yellow color or they may be crenated, or swollen and colorless (shadow cells). Blood cells are always pathological. Their source cannot be determined by appearance but may sometimes be suggested.

Sources of blood:

a. Acute nephritis and exacerbations of chronic nephritis. From an occasional cell to large amounts. Urine usually smoky colored.
b. Malignant disease of the kidney or bladder. Hemorrhage almost constant and may be profuse.
c. Renal tuberculosis. Blood is not constant but is often found.
d. Calculus, after the passage of a stone from the kidney or bladder.
e. Acute cystitis. Urine usually red.
f. Polypoid tumor of bladder. Often causes profuse hemorrhage.
g. Acute urethritis.
h. Traumatism from catheter.
i. Poisons, as turpentine, carbolic acid, cantharides and urotropine (occasionally after long administration).
j. Acute infectious diseases, as yellow fever, malaria and small pox.
k. Hemorrhagic diseases, as scurvy, leukemia, purpura and haemophilia.

3. *Pus cells.* About twice as large as red blood cells, round, granular, with irregular nuclei indistinct or invisible. They may be much or little degenerated. The addition of acetic acid to the slide will clear up the nuclei and make

their characteristics more distinct. An occasional leucocyte may be found in normal urine.

Sources of pus: Urethritis, cystitis, pyelitis, from various bacterial causes, including gonococci and tubercle bacilli. In nephritis a few pus cells may be present.

4. *Casts.* They are molds of kidney tubules. They are cylindrical, finger shaped bodies, of varying length. They appear when the kidney is affected by nephritis, circulatory changes or toxic irritants. The same causes which produce albuminuria cause casts. They are usually found together, though either may be found without the other.

The following varieties are recognized:

a. *Hyaline.* These are homogeneous and so transparent that the light must be considerably cut down in order to see them. They vary much in size, some being very small. They appear with less provocation than other varieties of casts, being found in temporary irritation of the kidneys, after anaesthetics, etc. They appear also in connection with other varieties in all forms of nephritis, and may be the only cast found in advanced interstitial nephritis.

b. *Granular.* The substance of this cast appears finely or coarsely granular and more or less dark looking. Sometimes the base of the cast seems to be hyaline with granules partly filling the clear substance. Granular casts indicate a definite lesion of the kidney and seldom appear except in nephritis. The coarsely granular cast has a more serious significance than the finely granular.

c. *Waxy.* Homogeneous, but less transparent and more refractile than hyaline. They are usually large, sometimes very long. Their color is sometimes slightly yellowish. They are of stiffer consistency than hyaline casts as shown by their ten-

dency to break off square at the end, and the fact that they are not seen to bend. They are sometimes twisted and often show transverse cracks. They appear in advanced nephritis and are of serious significance. In acute nephritis a cast called by many writers "fibrinous" is occasionally found which is difficult to distinguish from waxy. It is apt to be yellowish or brownish in color.

d. *Epithelial.* The cells of the tubules compose or cover these casts. Sometimes a hyaline or granular base appears. Sometimes the cast seems entirely formed of epithelial cells. They are found in acute and chronic nephritis and point to a serious lesion of the kidney.

e. *Blood cell casts.* These casts are covered by or formed of red blood cells. They appear whenever there is exudation of blood into the kidney tubules, oftenest in acute nephritis.

f. *Pus casts.* A few leucocytes may be attached to casts of any variety but true pus casts are formed entirely of pus cells. They are found in pyelonephritis, and are always of grave significance.

g. *Fatty casts.* These consist of masses of fat globules. They are the product of fatty degeneration of epithelial cells composing epithelial casts. If the degeneration is only partial and the cells can still be made out they usually are called epithelial. They appear in both acute and chronic parenchymatous nephritis.

Pus, blood and epithelial cells may all be found in the same cast. It will take its name from the predominating cells or be called a mixed cell cast.

Cylindroids are bodies of the apparent composition of hyaline casts, only less solid. One end appears to be molded

in a kidney tubule and the other end trails off into a narrow thread. They have but little significance, appearing often in normal urine as well as in company with hyaline casts.

Narrow, long, transparent, microscopic threads of mucus are a frequent finding in urine. Their source is the bladder and their significance nothing. These should not be confused with mucous shreds (tripperfäden) which are opaque flecks or strings seen by the naked eye, often a centimeter long, and when viewed under the microscope are seen to be thickly studded with leucocytes.

5. *Bacteria.* A few of the varieties which cause infection of the urinary tract can be identified without cultural methods, as the tubercle bacillus and the gonococcus. Normal urine is sterile but is a good culture medium, therefore all specimens not obtained and kept in an aseptic manner rapidly grow bacteria which have no significance but which cause cloudiness of the urine.

6. *Spermatozoa.* Spermatozoa may appear in the urine of adult males after intercourse or nocturnal emissions, as well as in cases of spermatorrhea and after epileptic or other convulsive attacks.

7. *Yeasts and moulds* are usually contaminations, but have been known to infect the bladder in cases of diabetes, the saccharine urine furnishing a favorable medium for their growth.

DIAGNOSTIC URINARY FINDINGS IN CERTAIN DISEASES

	PHYSICAL	CHEMICAL	MICROSCOPICAL.
Acute Nephritis	Small amount, highly colored. Cloudy or smoky. Specific gravity high.	Large amount of albumin. Normal solids diminished.	Many casts of all varieties especially blood and epithelial blood cells few or many. A few leucocytes.
Chronic Parenchymatous Nephritis	Small or normal amount. Normal or dark in color, cloudy. Specific gravity high or normal.	Very large amount of albumin. Normal solids diminished.	Many casts of all varieties, especially granular, fatty and waxy. Occasional blood cells. A few leucocytes.
Chronic Interstitial Nephritis	Large amount, light color, clear. Specific gravity low.	Albumin present, often in small amounts. May be temporarily absent.	Hyaline and granular casts. May be but few. Occasional red blood cells.
Diabetes Mellitus	Very large amount. Color very light, may be greenish, clear. Specific gravity very high.	Sugar present. Acetone bodies in severe cases.	
Diabetes Insipidus	Very large amount. Color very light, clear. Specific gravity very low.	No albumin nor sugar. Normal solids in normal amounts in 24 hr. output.	
Renal Tuberculosis	Amount normal or large. Cloudy, acid reaction.	Albumin present, a trace or more.	Pus in small or large amounts. Blood may be present. Tubercle bacilli.
Renal Calculus	Smoky, cloudy or normal in appearance	Albumin present, usually a trace.	Red blood cells, few or many. Pus cells if there is pyelitis. Crystals of calcium oxalate. Uric acid or cystin.

CHAPTER IV.

STOMACH CONTENTS.

Test Meal.—

A specified test meal removed at a definite time is necessary so that the findings may be comparable. The Ewald breakfast is usually used in this country and consists of 35 grams of bread (2 slices) and 400 c.c. (2 cups) of water or clear weak tea. The test meal is given on an empty stomach and the stomach contents are then removed in 1 hour.

Stomach contents after a test meal normally consist of
1. *acids;* hydrochloric acid, free and combined,
2. *ferments*; pepsin or pepsinogen, rennin or rennin zymogen;

3. *food products;* starch and products of starch digestion, i. e., erythrodextrin and achroodextrin; proteids and products of proteid digestion, i. e., acid albumin, albumose and peptone; and acid salts.

Pathological substances which may be present are lactic acid (more than a trace), mucus (excess), blood, bile, pus, bacteria (large numbers), yeasts and moulds.

Examination of Stomach Contents After Ewald Test Breakfast.

I. Macroscopical Examination.—

Observe amount, color, odor, character of food particles

whether coarse or finely divided, consistency whether watery or viscid, mucus whether mixed with food or floating. If mucus is in excess and mixed with the food the stomach contents will string out when poured from one container into another.

II. Microscopical Examination.—

Place a drop of unfiltered stomach contents on a slide, add a drop of Lugol's solution, mix, cover with cover glass and examine with high dry lens. Starch granules are stained blue or violet, epithelial cells, pus cells, yeast cells and bacteria, yellow, and fat droplets are unstained.

III. Chemical Examination.—

Filter and use the filtrate for the chemical examinations.

1. Titration.

Measure 5 c.c. of filtrate into each of two flasks. Dilute each to 25 c.c. with distilled water.

To the first flask add 3 drops of phenol phthalein (1 % alcoholic solution) and 1 drop of dimethylamido-azo-benzol (0.5 % alcoholic solution). If free HCl is present, solution turns red; if free HCl is absent solution turns yellow. To the second flask add 1 drop of alizarin (1 % aqueous solution). If free acid is present solution turns bright yellow, if absent solution turns violet.

Titrate mixture in flask No. 1 with N/10 NaOH from burette until red color changes to orange. Take burette reading. Number of c.c. N/10 NaOH used X20 indicates degree of acidity due to free HCl. Continue titration until first permanent pink color appears. Take burette reading. Total number of c.c N/10 NaOH used, including the amount used in first determining the free HClX20 indicates the degree of total acidity.

Titrate solution in flask No. 2 until yellow color changes to violet. Take burette reading. Number of c.c. N/10 NaOH used X20 subtracted from degree of total acidity indicates degree of combined HCl.

The sum of the free and combined HCl subtracted from the total acidity, gives the degree of acidity due to acid salts and organic acids.

The acidity of stomach contents is usually expressed in degrees, degree of acidity meaning the number of c.c. of N/10 NaOH required to neutralize the acidity of 100 c.c. of stomach contents.

Phenol phthalein indicates total acidity. Dimethyl-amido-azo-benzol (Töpfer's Reagent) indicates free HCl. Alizarin indicates all acidity except that due to combined HCl.

2. Test for Ferments.—

If hydrochloric acid is absent test for ferments. Pepsin and rennin run parallel, therefore the test for rennin being simpler is sufficient for both.

Pour 5 c.c. of milk into each of 3 t. t. and add 2 c.c. of 1 % solution calcium chloride to each. Dilute 1 c.c. of filtrate from stomach contents with 9 c.c. of water, and add 5 c.c. of this dilution to tube 1. To the remainder of the diluted filtrate add 5 c.c. of water, and add 5 c.c. of this second dilution to tube 2. Incubate the three tubes ½ hour.

If rennin is present in normal amount the milk in tubes 1 and 2 will be coagulated. If the milk is not coagulated in either tube rennin is absent. If the milk is coagulated in tube 1 and not in tube 2, rennin is deficient.

3. Test for Lactic Acid.—

Lactic acid is never found in the presence of free HCl, hence test for it only when free HCl is absent.

Kelling's Test. To a t. t. full of distilled water add ferric chloride solution to just color the water. Divide into 2 t. t.

Add filtrate from stomach contents drop by drop to one of the tubes, the second tube being used as a control. If lactic acid is present a canary yellow color will appear as drops are added.

4. Test for Blood.—

Weber's Test. Add 1/3 volume glacial acetic acid to unfiltered stomach contents (about 10 c.c.) and shake. Add about 5 c.c. ether and gently invert tube a few times. Add 1 c.c. of fresh tincture of guaiac (alcoholic solution of gum guaiac), and excess of hydrogen peroxide (about 2 c.c.). If blood is present a blue ring will appear at the line of contact of ether and stomach contents spreading upward throughout the ether. The depth of color indicates roughly the amount of blood present.

5. Test for Peptone.—

Add a few drops of Haines' solution to about 5 c.c. of filtered stomach contents. A violet color will appear if peptone is present. Peptone is present if combined HCl is present.

6. Test for Starch.—

Add Lugol's solution drop by drop to about 5 c.c. of filtered stomach contents. A blue color will appear if unchanged starch is present, a brownish color if erythrodextrin is present and no change in color or an absorption of the color of the Lugol's solution if achroodextrin is present. Normally after the addition of the first drops of Lugol's solution there is some absorption of color due to the presence of achroodextrin followed later as more Lugol's is added by the appearance of the brownish color due to erythrodextrin.

Normal Stomach Contents After Ewald Test Breakfast.

Macroscopic:

Amount, less than 150 c.c.
Color, grayish white to yellow.
Character of food particles, finely divided.
Odor, sour.
Consistency, semi fluid, separates into two layers.
Mucus, little or none mixed with food.

Chemical:

Total acidity, 40°-60°.
Free HCl, 20°-40°.
Combined HCl, 10°-20°.
Organic acids and acid salts, less than 10°.
Pepsin and rennin present.
Peptone present.
Erythrodextrin and achroodextrin present.
Lactic acid absent.
Blood absent.

Microscopic:

Few bacteria. No Boaz-Oppler bacilli, sarcinae or yeasts. Few epithelial cells. No pus or blood cells. Mucus from the throat is often found in the stomach contents and shows numerous leucocytes and epithelial cells. Numerous starch granules stained blue or violet with Lugol's solution.

Pathological Variations.

Amount.—Is increased in hypersecretion and motor insufficiency.

Color.—Blood may give a red, brown or black color. Green color may be due to bile or green algae.

Character of Food Particles.—Coarse when HCl is deficient or absent. Remnants of former meals found in stasis.

Consistency.—Watery in hypersecretion, thick and viscid when excess of mucus is present.

Mucus.—If intimately mixed with food is pathognomonic of mucous gastritis. If in lumps or floating in liquid portion, it is swallowed mucus.

Free HCl.—Increased in hyperacidity and most cases of gastric ulcer. Decreased in acute gastritis, chronic gastritis, and general systemic depression. Absent in most cases of carcinoma, advanced chronic gastritis and pernicious anemia.

Lactic Acid.—Present only in absence of free HCl. Its presence is the most valuable single diagnostic symptom of gastric cancer.

Ferments.—If ferments are absent it is due to actual destruction of the secreting glands. They are seldom absent except in atrophic gastritis and carcinoma.

Starch Digestion.—Hyperacidity inhibits starch digestion, therefore in hyperacidity unchanged starch is present. If HCl is diminished or absent, starch digestion proceeds to achroodextrin.

Proteid Digestion.—Proteid digestion is poor when there is no free HCl and little combined HCl. Little or no proteid digestion takes place if total acidity is very low. Proteid digestion is rapid in hyperacidity.

Blood.—Usually present in carcinoma (coffee ground). Hemorrhages occur in many cases of ulcer. Occult blood (chemical, not visible) at times in ulcer, as a rule in carcinoma. Gastric hemorrhages may occur (a) in primary disease of the stomach as ulcer or cancer, (b) secondary

disease of other organs as in chronic passive congestion and cirrhosis of the liver.

Bacteria.—Large numbers of bacilli, micrococci and yeast cells are present when there is stasis. Yeasts and sarcinae are present in gastric dilatation of benign origin. In gastric carcinoma large numbers of large, long bacilli, the Boaz-Oppler bacilli, are present.

DIAGNOSTIC CHARACTERISTICS OF STOMACH CONTENTS IN CERTAIN DISEASES.

ACUTE GASTRITIS	CHRONIC GASTRITIS	HYPERACIDITY	ULCER	CARCINOMA
Total acidity low.	Food particles coarse.	Consistency watery. Food particles very fine.	HCl increased in about 50% of cases. May be normal or diminished.	Food particles coarse, food remnants of previous meals (if obstruction), "coffee ground" color if visible blood.
HCl low or absent.	TOTAL ACIDITY LOW. May be less than 10°	TOTAL ACIDITY INCREASED. May be 100°+	BLOOD intermittent either occult or visible.	Amount depends on pyloric stenosis. Increases as motor power is interfered with...
Mucus present.	HCl DIMINISHED or absent	HCl INCREASED 50°+		HCl usually absent.
Ferments present.	MUCUS, large amount mixed with the food.	Starch digestion inhibited.		LACTIC ACID present.
	Ferments diminished or absent depending upon the stage of the gastritis.	Proteid digestion good.		BLOOD nearly constant, occult or visible.
	Starch digestion very good.	Yeasts and sarcinae present with motor insufficiency from benign obstruction.		Mucus present if there is accompanying gastritis.
	Proteid digestion poor.			Ferments diminished or absent according to accompanying gastritis.
				BOAZ-OPPLER BACILLI present in large numbers.

CHAPTER V.

FECES.

Macroscopical Examination.—

Observe color, whether "tarry," red or streaked with blood, whether colorless, glistening gray or clay-like from excess of fat or absence of bile. Normally there is a great variation in color dependent chiefly upon the diet.

Observe the excess of mucus, whether in the form of shreds and flakes mixed with feces, or in ribbon like strips, or mixed with blood or pus.

Examine washed feces for stones, connective tissue and parasites. To wash feces, place a small amount in a large beaker, add water, stir, let settle, pour off water and repeat process several times.

Microscopical Examination.—

Make three separate mounts of feces, (1) one drop of feces mixed with water for examination of food remnants, blood, pus, ova and parasites as protozoa, (2) one drop of feces mixed with one drop of Lugol's solution for identification of starch granules, (3) one drop of feces mixed with one drop of Sudan III (70% alcoholic solution) for identification of fat. Examine under both low power and high dry lens.

Food Remnants.

1. Muscle fibres. Nearly always present. Appear as

cylindrical yellow fragments, somewhat rounded, striated or homogeneous according to the degree of digestion.

2. Vegetable detritis. Always present and generally in the form of plant cells with a distinct cell wall, commonly starch masses enclosed in a cellulose membrane, thorn like spines from fruits or berries, spiral cells (veins of leaves), pitted ducts. The background is made up of masses of bacteria and formless debris. Free starch granules are stained blue or violet with Lugol's solution.

3. Neutral fat. Droplets or masses with irregularly rounded outlines stained red with Sudan III.

4. Fatty acids. Small formless bile stained masses, or colorless delicate needle like crystals, often in sheaves.

5. Soaps. Angular amorphous masses usually bile stained, or colorless short broad needle like crystals, often in sheaves.

Mucus, Blood, Pus.

Microscopically mucus appears as more or less transparent masses with faintly marked outlines, usually mixed with food detritis, bacteria, leucocytes, epithelium and is often bile stained. Epithelium, leucocytes and blood cells are usually so altered and mixed with the feces they are hard to identify unless present in excess with mucus as in catarrhal, ulcerative, or dysenteric processes.

Parasites and Ova.

In examining for protozoa the feces should be fresh, the spread of feces on the slide thin and the slide warmed or kept on a warm stage in order to observe the movements of the parasites. The general recognition of the parasites, their movements, etc., and of ova is done best under low power and further identification made under high dry lens.

An amoeba should not be diagnosed unless its motility by pseudopodia can be demonstrated. At rest it resembles a large epithelial cell. Trichomonas intestinalis and lamblia intestinalis often associated with amoebae are very motile, darting about by means of flagellae, and are about 3 times the size of a red blood corpuscle.

Most ova are oval in shape, yellow to brown in color, average about 50x30 μ in size (size of many vegetable masses in feces), possess a definite shell and a central protoplasm which may be granular, segmenting or may contain an embryo. Vegetable cells are often mistaken for ova. The very definite form of the ova with their clean cut contour, the structure and thickness of their shells are usually sufficient to identify them.

Chemical Examination.—

Weber's Test for Blood.

To about 5 c.c. of feces in a large test tube add ⅓ volume of glacial acetic acid, cork and shake tube. Add 10 c.c. of ether and invert the tube gently 3 or 4 times. To ether extract add 0.5 c.c. of fresh tincture of guaiac and 2 c.c. of hydrogen peroxide. A blue color indicates blood. If fat is present in excess extract with ether several times before applying the test.

If the feces has given a positive reaction or if occult gastric or intestinal blood is suspected it is best to put the patient on a meat free diet for a few days. If the reaction is then positive the blood is pathological.

Pathological Findings and Significance.—

Mucus.

Any amount of visible mucus is abnormal. The amount seen pathologically varies enormously. It may be seen in the form of:

a. Shreds, lumps, small flakes, somewhat homogeneous and transparent, rich in cells and detritis of digestion, varying in amount from small portions to nearly pure mucus, as in acute and chronic catarrhal enteritis.

b. Large amounts of mucus mixed with blood and pus, as in dysentery.

c. Strips of tough leathery mucus from the large bowel as in conditions of secretory neurosis.

Blood.

The feces may be red or "tarry" from the presence of blood, may contain microscopic blood or occult blood, the color of the blood containing feces depending upon the amount and source of blood.

1. Blood in streaks on formed stool points to hemorrhoids or rectal fissure.

2. "Tarry stool." Blood usually comes from stomach or duodenum. Blood in large amounts from small intestine with increased peristalsis may appear red as in hemorrhage from typhoid or duodenal ulcer.

3. Occult blood. Continuous in malignant diseases of the alimentary tract. Occasional and intermittent in peptic or duodenal ulcer.

4. Blood mixed with pus and mucus in dysentery.

Pus.

Pus in feces is usually indicative of ulceration. Large amounts of pus may appear in case of ruptured extra-intestinal abscesses, extensive ulcerated carcinomata of colon or rectum, and dysentery. Small amount of pus is present in the feces in the majority of cases of simple ulcer.

Fat.

A clay-like stool usually contains fat droplets and large masses of fatty acid crystals.

An excess of fat in the feces may occur when there is:

a. Increased peristalsis.

b. Interference with fat absorption in small intestine as in amyloid degeneration of the intestine, tuberculosis of the intestine, chronic tubercular peritonitis, tabes mesenterica, cancer of the intestine.

c. Biliary obstruction.

d. Pancreatic disease.

Stones.

1. Gall stones. These may be found in the feces after the colic in cholelithiasis. They are friable, yellow or brown, smooth or facetted, and vary much in size. They are composed of bilirubin, calcium and cholesterin, and show concentric layers when fractured.

2. Pancreatic stones (rare). In size no larger than a pea, usually single, colorless, irregular in shape, composed of calcium carbonate and calcium phosphate.

3. Enteroliths or fecal concretions (rare). Undigested masses impregnated with calcium and magnesium phosphate, very hard, may reach the size of large egg.

DISTINGUISHING CHARACTERISTICS OF CERTAIN INTESTINAL PARASITES.

NAME	FORM, SIZE, ETC.	HABITAT	LIFE CYCLE CHARACTERISTICS.	INFECTION	DIAGNOSIS.	OVA.
Ascaris Lumbricoides (Stomach Worm)	Round worm 4-10 inches long. Brownish color, characteristic odor.	Small intestine, may wander	Adult worm develops in same form from ovum.	Ova.	Ova in feces. May find worm in feces.	Dark reddish brown shell with rough surface.
Oxyuris Vermicularis (Pin worm or seat worm)	Round worm, ¼ inch long.	Large and small intestine.	Embryo set free from ovum in small intestine, becomes sexually mature as it passes along the intestine. Pregnant females in colon.	Ova.	Worms in feces. Ova about the anus.	Thin colorless shell. One surface flattened. Protoplasm in stages of embryonic development.
Uncinaria duodenale. Anchylostoma duodenale (Hook worm)	Round worm, ½ inch long. Whitish or blotched with brown. Hooklets about mouth.	Small intestine.	Ova develop in dirt or feces outside the intestine to larval form. Larval form enters intestine, continues development in small intestine and attaches itself to mucous membrane.	Larvae enter intestine through food or dirt or burrow through skin.	Ova in feces.	Colorless, thin shell. Protoplasm granular and segmenting into 2, 4, 8 or more rounded segments.
Trichocephalus trichiuris (Whip worm)	Round worm, 2 inches long. Ant. end like thong of whip. Post. end like handle of whip.	Large intestine.	Adult worm develops in same form from ovum.	Ova.	Ova in feces. Worm rarely in feces.	Brown thick shell. Button projection at each end.
Strongyloides intestinalis	Embryos ¼—½ mm. Cylindrical, tapering to tail.	Small intestine.	Life history complicated. From ova develop the rhabditiform embyro which generally passes into filariform embryo which develops into pathenogenetic females (strongyloides intestinals) and sexually mature (Rhabditis stercoralis.)	Little known. Probably through embryo.	Rhabditiform or filariform embryos in feces. Ova and adult forms rare.	

Trichina spiralis	Round worm adult form, 3-4 mm. Embryo 1 mm.	Larval stage (trichina) in muscle. Adult stage in intestine.	Larvae develop in intestine to adult form which gives birth to embryos which migrate through blood to striated muscle where they become encapsulated.	Larvae or "muscle trichinae" in muscle of hog.	Rare to find adult form or embryo in feces Trichinae in muscles. Leucocytosis with eosinophilia.	
Taenia solium (Pork tapeworm) Taenia saginata (Beef tapeworm)	Pork tape worm. Flat worm, 9 feet long. Head (scolex) smaller than head of pin, has 4 suckers and circlet of hooklets. Beef tape worm. Flat worm. 12-20 feet long. Scolex size of head of pin, has 4 sucking discs and no hooklets.	Adult form in intestine of man only.	Ova develop in uterus of worm to embryo with scolex and hooklets. Ova enter stomach of hog or beef. Shell is dissolved and embryo bores through intestinal wall and becomes embedded as cysticercus in muscles. Muscle ingested by man Scolex of embryo attaches itself to the intestinal wall and develops into adult tape worm.	Pork containing cysticercus or embryo. Beef containing cysticercus or embryo.	Segments and ova in feces.	Round in form. Shell thick and radially striated.
Taenia ecchinococcus	Flat worm, 2-5 mm. long. 4 segments. Head has double row of hooklets and 4 sucking discs.	Adult form in intestine of dog.	Ova reach the digestive tract of man where embryos are set free and find their way to liver, lung, brain or other organ where multilocular cysts develop.	Ova.	Scolices, hooklets or portions of cyst wall in cyst fluid, sputum, feces or urine.	

CHAPTER VI.

HUMAN MILK.

Composition of Normal Human Milk.

Reaction—slightly alkaline or neutral.
Sp. Gr.—1028-1032.
Fat—3 to 5%.
Lactose—6 to 7%.
Protein—1 to 2.25%.

Microscopically, fat droplets are present and during the first few days of lactation, colostrum corpuscles (large granular cells).

Variations in the amount of fat, proteid and sugar are the chief changes which have a practical bearing on infant feeding.

Fat.

Use Holt's cream gauge. Fill cream gauge to zero mark with fresh milk. Let stand 24 hours. Read % of cream. The ratio of cream to fat is as 5 to 3. Thus 5% cream indicates 3% fat.

The fat content may be determined immediately with the small Babcock tube which fits the cup of the ordinary centrifuge. 5 c.c. of milk is pipetted into the tube, 5 c.c. of concentrated H^2SO^4 is added a little at a time, mixing with each addition, and finally enough of a mixture of equal parts of concentrated HCl and amyl alcohol is added to fill

the tube. Centrifuge the tube 5 minutes and read the percentage of fat directly on the tube.

Protein.

Boggs' Modification of the Esbach Method.

Dilute the milk ten times and fill Esbach albuminometer to mark U with diluted milk. Add the reagent to the mark R. Mix by turning. Let stand 24 hours. With this dilution the marks on the tube give the percentage of the protein.

Boggs' Reagent.

Phosphotungstic Acid .....25 grams.
Concentrated Hydrochloric Acid.....25 c.c.
Distilled water q. s. ad.....250 c.c.

Lactose.

Mix equal parts of milk and Boggs' Reagent, dilute with equal volume of water, filter and estimate lactose in the filtrate by the titration method as in the quantitative estimation of sugar in urine. 0.014 grams of lactose reduces 10 c.c. of Haines' quantitative solution.

To calculate: 0.014 divided by the number of c.c. of undiluted milk used multiplied by 100 gives the % of lactose.

CHAPTER VII.

CEREBRO-SPINAL FLUID.

Normal cerebro-spinal fluid is limpid and colorless and contains very few cells. It contains enough sugar to reduce copper solutions and a very slight trace of proteid material.

Bacteriological Examination.

Make smears from centrifuged sediment of spinal fluid. Stain with Gram, also with carbol fuchsin if tubercle bacillus is suspected. Make cultures of centrifuged sediment on blood agar and blood serum, using culture tubes and Petri dishes in which blood agar has been poured. In smears and cultures examine for meningococcus intracellularis, tubercle bacillus, pneumococcus, streptococcus, typhoid bacillus, colon bacillus and influenza bacillus. Other bacteria have been reported but rarely. In examining for tubercle bacilli in spinal fluid it is best to allow the fluid to stand 24 to 48 hours to allow a pellicle to form. This pellicle may contain the bacilli when they cannot be found in the fluid proper.

Cytology.

Differential Cell Count.

The fluid is centrifuged, the sediment smeared, dried and fixed on a slide and stained with Wright's blood stain. A differential count of the cells is then made as a differential

leucocyte count is made. In the acute infections the polymorphonuclear cells predominate and in the chronic infections as tuberculosis and syphilis the lymphocytes predominate.

Total Cell Count.

In fluids containing few cells (clear fluids) the number of cells per cu.mm. is estimated as follows: The leucocyte pipette is filled to the 1 mark with 10% acetic acid, the fresh spinal fluid, well shaken, is then drawn up to the mark 11. The mixture is shaken and a drop mounted in the counting chamber as in counting blood. The Türck counting chamber is used and the cells in the whole ruled space, 9 sq. mm., are counted. This space contains 9/10 cu. mm., of fluid. The mixture is 9/10 spinal fluid and 1/10 diluting fluid. Therefore the number of cells counted X10/9X10/9= the number of cells per cu. mm. For example the 9 sq. mm., contains 40 cells. 40X10/9X10/9= 49+. If cells are so numerous as to cause clouding, the spinal fluid must be diluted as for a leucocyte count of the blood. The number of cells per cu. mm. of normal spinal fluid is less than 10.

Chemical.

Test for Globulin.—

The Ross-Jones modification of the Nonne test for excess of globulin is as follows: 1 c.c. of spinal fluid is carefully layered upon 2 c.c. of saturated solution of ammonium sulphate in a small test tube. A grayish white ring at the contact, appearing within a few minutes, is a positive reaction and indicates a pathological amount of globulin in the spinal fluid.

The test is positive in all infections of the nervous system and negative in normal spinal fluid.

The Lange Colloidal Gold Reaction.

Technique of the Test.

Ten test tubes (6 in. x ¾ in.) are placed in a row in a test tube rack. To the first add 1.8 c.c. of a freshly made 0.4% sodium chloride solution. To each of the other tubes add 1 c.c. of the same solution. Draw up into a 1 c.c. pipette 0.2 c.c. of the spinal fluid to be tested and add it to the first tube. This makes a dilution of 1-10 in tube 1. From the first tube remove with the same pipette 1 c.c. of the mixture and place it in tube 2. This will make a dilution of 1 in 20 in tube 2. Remove 1 c.c. from tube 2 and place it in tube 3, making a dilution in tube 3 of 1 in 40. Continue in this way to the end of the series, discarding the 1 c.c. removed from tube 10. The dilution in each tube is double that in the tube before it, the dilution in tube 10 being 5120. Add to each tube in the series 5 c.c. of the colloidal gold solution. At the end of twenty-four hours a final reading is made, although strong reactions will show in a much shorter time.

The reaction consists in color changes due to more or less precipitation of the colloidal gold. The solution itself is red and clear. Slight precipitation gives a bluish tint to the fluid. Increasing amounts of precipitation change it to violet blue, grayish and when precipitation is complete, colorless. The amount of change is expressed by numbers, 5 representing complete precipitation and the least observable change 1. A negative reaction would be written 0000000000. One in which precipitation is complete in the first four tubes and less in the fifth, sixth and seventh would be written 5555431000. Preparation of the colloidal gold solution used as indicator requires great care and is often unsuccessful in inexperienced hands. Specific directions for its preparation with discussion of its difficulties may be found in Bull. Johns Hopkins Hospital xxvi,

1915, p. 391. The colloidal gold solution can be purchased from E. H. Sargent.

Diagnostic Value.

The diagnostic value of the test is considerable as it distinguishes several types of cerebro-spinal disease.

In general paresis complete precipitation occurs in the lower dilutions, the typical reading being 5555431000. Complete precipitation may occur in even higher dilutions.

In cerebro-spinal syphilis and tabes the greatest color change occurs in the third and fourth tubes, a typical reading being 1133200000.

In non-syphilitic meningitis the greatest precipitation occurs in the higher dilutions, that is, beyond the fourth tube.

In normal spinal fluid the color of the whole series remains unchanged.

CEREBRO-SPINAL FLUID

DISEASE	APPEARANCE	GLOBULIN.	SUGAR	NO. OF CELLS.	DIFFERENTIAL CELL COUNT	BACTERIA
Acute Meningitis	May be clear, often cloudy, coagulates on standing.	Much increased.	Absent.	Very high	Polynuclears predominate	Meningococcus Pneumococcus Streptococcus Influenza B, etc.
Tubercular Meningitis	Clear. Delicate pellicle on standing.	Increased.	Present. May be less than normal.	Increased.	Mononuclears predominate	Tubercle bacillus.
Syphilitic Diseases of Nervous System	Clear	Usually increased.	Normal.	Increased.	Mononuclears predominate	Spirochaeta Pallida
Normal	Clear and colorless.	Slight trace. Negative Nonne.	Present. Reduces Fehlings.	Less than 10 per c.mm.		Absent.

CHAPTER VIII.

SPUTUM.

Macroscopic.—

The consistency or character of the sputum depends upon the presence and relative amount of mucus, serum, pus or blood present. Mucoid, serous, muco-purulent, purulent and sanguinous are terms descriptive of the character of the sputum as determined by its leading constituents. The more mucus there is the more tenacious the sputum. The color due to blood may be the color of fresh blood or that of the haemoglobin derivatives.

Formed bodies which may be recognized macroscopically:

a. Curshmann's spirals, twisted threads of mucus, characteristic of bronchial asthma.
b. Dittrich's plugs, foul smelling, cheesy, cylindrical masses, found in chronic bronchitis.
c. Elastic fibres and necrotic tissue found in advanced tuberculosis or other destructive diseases of the lung.
d. Molds of the bronchi, found in fibrinous bronchitis.
e. "Sulphur granules" found in actinomycosis.

Microscopic.— .

Unstained. A loopful of the sputum is put on a slide, covered with a large cover glass and examined with high dry lens.

The ray fungus of actinomycosis, blastomycetes, hooklets of echinococcus, entamoeba histolytica and pigmented epithelial cells and crystals are best seen in the unstained specimen.

Stained. The most important information to be gained from the stained specimens is the demonstration of the tubercle bacillus, and other pathogenic bacteria. The standard method for the demonstration of the tubercle bacillus in sputum is the Ziehl-Neelsen Method, which is described under stains in the chapter on bacteriology. A Gram stain should be made for the examination of bacteria other than the tubercle bacillus. The chief pathogenic bacteria to be looked for are the pneumococcus, the influenza bacillus, Friedlander's bacillus, micrococcus catarrhalis and streptococcus. When any of these bacteria are the causative agent of an infection they will be present in large numbers in the sputum and will be the predominating bacteria.

Sputum in Certain Diseases.—

Pulmonary Tuberculosis.

Tubercular sputum is usually purulent, but sputum of any character may be found in this disease. The presence of the tubercle bacillus is the diagnostic feature. The more advanced the destructive process in the lung, the greater the number of tubercle bacilli present.

Acute Lobar Pneumonia.

Early, the sputum is scanty, tenacious, translucent and rusty colored. Toward the crisis it becomes more abundant, muco-purulent and later during resolution becomes mucoid. If blood continues to be present, as it often does, the sputum is colored dark by it. The pneumococcus is present in large numbers in those cases caused by the pneumococcus.

Acute Bronchitis.

Sputum is at first scanty, mucoid, soon becomes abundant muco-purulent, then purulent, sometimes blood streaked.

Chronic Bronchitis.

Sputum usually abundant, muco-purulent, may vary much in character and amount.

Asthma.

Often there is no sputum during the paroxysm; if present it is scanty, clear, consisting of thick glairy mucous balls. Curshmann's Spirals, Charot-Leyden crystals, and eosinophiles are characteristic.

Pulmonary Oedema.

Large amounts of frothy, serous, blood stained sputum.

Abscesses, Gangrene, Bronchiectasis, Tubercular Cavities.

The intermittent appearance of large amounts of purulent sputum, often fetid, containing necrotic tissue, and crystalline products of decomposition is characteristic.

CHAPTER IX.

BACTERIOLOGY.

Methods of Bacteriological Examination of Pathological Material.

The bacteriological examination of pus or other material consists of (1) the examination of stained smears to determine the morphology of the bacteria, the staining reactions, the spore and capsule formation, the number of bacteria and the distribution of the bacteria, whether intra or extracellular; (2) the cultural study to isolate the different varieties of bacteria in pure culture and to identify them by cultural characteristics as to the appearance and luxuriance of growth on the different media, the production of chemical changes in the media such as the production of acid in milk, the fermentation of sugars with acid or gas formation, the production of indol, the liquefaction of gelatin, of coagulated blood serum or of casein; (3) the serum reactions as the agglutination and complement fixation tests and (4) animal inoculation.

Smears and cultures should be made directly from the patient as far as possible. When material is to be carried to the laboratory to be examined bacteriologically, whether in liquid form, tissue or on cotton swabs, it should be placed in dry sterile bottles. In aspirating pus or fluid, for example from tooth sockets, it is convenient to use capillary pipettes. These are made by drawing out tubing of the diameter used for the ordinary medicine dropper to capillary fineness of about 1 mm. They may be sterilized in the sterilizer or

in the flame. The capillary end is sealed in the flame after the fluid is aspirated, and when ready for examination, is broken off and pus blown out on slides for smears and on the surface of media in Petri dishes and tubes for cultures.

When only a small amount of pus is available platinum loops or cotton swabs (wooden applicators wound at the ends with a bit of cotton, sterilized in hot air sterilizer and stored in cotton plugged tubes) are more practical. Smears and cultures are made directly from the loop or swab.

In examining extirpated *tonsils* make smears and cultures with a platinum loop from the depth of the crypts; lay open the tonsils with a sharp sterile knife and make smears and cultures from the inside. The tonsil can be ground in a sterile mortar with a small amount of sterile salt solution after the surface baceria have been killed by plunging the tonsil into boiling water. Cultures are made from this salt solution emulsion. Extirpated *glands* may be ground up in salt solution and examined in the same way. *Urine,* preferably a catheterized specimen, is centrifuged in sterile centrifuge tubes, the supernatant fluid poured off and the few drops of sediment used to make smears and cultures. *Sputum* collected in sterile bottles should be examined preferably soon after expectoration. Smears and cultures should be made directly from the purulent portions of the sputum and also after washing the sputum in several changes of sterile water to remove the surface mouth bacteria. *Blood* for bacteriological examination is best obtained from one of the large veins at the bend of the elbow. The skin is thoroughly cleansed with alcohol, a constrictor applied to the upper arm, a sterile needle (large size, about No. 19) attached to a sterile syringe inserted into a vein and about 10 c.c. of blood withdrawn into the syringe. The needle is then removed and the blood distributed into flasks of glucose bouillon in the proportion of one part of blood to about 50 parts of bouillon. If growth takes place in the bouillon as shown by the microscopical examination of

stained smears from the coagulum, transplants should be made on other media to identify the bacteria.

Smears.

For the making of smears one should have at hand slides, and a platinum loop or cotton swabs. A small loopful of the material to be examined or the cotton swab moistened with it is spread over a glass slide in a thin film, a loopful of water being added when necessary to aid in making the film thin. The smears are fixed in the flame and separate slides stained with methylene blue, Gram and carbol fuchsin. If one slide only is used, Gram's stain is preferred. When several different films are to be stained in the same way parallel films can be made on one slide by using a small amount and stroking crosswise instead of lengthwise of the slide.

Culture Media and Making of Cultures.

The smears give some information as to the number and varieties of bacteria so that one can be guided as to the special media needed in making cultures and the amount of material to be used in inoculation. Culture media needed for general examinations:

1. Agar agar—Petri dish and slant.
2. Loeffler's blood serum—slant, aerobic and anaerobic.
3. Blood agar—Petri dish and slant, aerobic and anaerobic.
4. Ascitic glucose agar—slant and deep tube.
5. Ascitic glucose bouillon.
6. Glucose bouillon—fermentation tube.

It is not always necessary to use all of these different kinds of media, but where possible it is best to do so as it gives a better chance to grow any bacteria present and a better separation of the colonies. If all media are not available use preferably blood agar and blood serum aerobically and anaerobically.

Loeffler's blood serum.—

Mix one part of dextrose bouillon with three parts of beef serum, tube and sterilize the tubes on a slant in a blood serum inspissator or Arnold steam sterilizer on three successive days.

Blood agar.—

Melt about 10 c.c. of neutral or slightly alkaline glucose agar, cool to 50° C., add ½ to 1 c.c. of defibrinated blood (human or goat), mix and pour in sterile Petri dish to harden. Slant blood agar is made in the same way, slanted and allowed to harden.

Ascitic glucose agar.—

Melt glucose agar, cool to 45° or 50° C., add sterile ascitic fluid in the proportion of one part ascitic fluid to 3 parts of agar. Pour in Petri dish, slant in tube, or use for deep tube inoculation.

Edno medium. (Kendall's modification.)

1% lactose agar slightly alkaline to litmus, sterilized and stored. When needed add 1% decolorized fuchsin (1 c.c. sat. alc. sol. fuchsin+10 c.c. of 10% watery sol. sodium sulphite), pour in plates, allow to harden and inoculate by streaking.

Bile Medium. (For typhoid blood cultures.)

Add 10% of glycerine and 2% of peptone to ox bile. A 10% solution of dried fresh ox gall can be used in place of the ox bile. The medium is sterilized in the autoclav and stored in flasks, 25 c.c. to a flask.

In making a blood culture add 1 part of blood to 3 parts of bile medium.

Inoculation of Media in Petri Dish.

When the medium is sufficiently firm usually in 15 or 20 minutes after pouring make many strokes across the plate

with the platinum loop or swab dipped in the material to be examined. There will be a thick inoculaton under the first needle strokes and a thinning out of the bacteria under the last strokes, so that well separated colonies will appear. Incubate the plates with cover side down to prevent the water of condensation from mixing the colonies.

Anaerobic Cultures.

Anaerobic cultures are conveniently made on Loeffler's blood serum and slant blood agar, but may be made on any media. After inoculation the cotton plug is pushed down to the level of the top of the medium slant, the tube filled (as capsules are filled) above the cotton with pyrogallic acid to the depth of about 1 inch, 5 to 10 drops of 5% sodium hydroxide are added and a cork previously soaked in paraffin put in tightly. Lay the tubes in the incubator on a slight slant with the cork end downward. If anaerobic cultures are to be made in liquid media the same method is used, but an extra cotton plug is necessary to prevent the sodium hydroxide from working its way down to the medium, and the tube must be kept upright in the incubator. To inoculate deep tubes of ascitic glucose agar, melt the agar, add the ascitic fluid as previously described, then inoculate the medium while still fluid, mix well and let solidify. When solid make anaerobic in the same manner as slant blood agar tubes.

Stains and Methods of Staining.

Methylene Blue Method.—

1. Fix smear in flame.
2. Cover with methylene blue ½ minute.
3. Wash in water and dry between filter paper.

Loeffler's Methylene Blue. (Wright's formula.)

Methylene blue	0.5 gram.
Sodium carbonate	0.5 gram.
Water	100 c.c.

Gram's Method.—

1. Fix smear in flame.
2. Stain ½ minute in anilin gentian violet.
3. Wash in water.
4. Cover with Gram's iodine ½ minute.
5. Drain and drop on 95% alcohol until alcohol runs off clear.
6. Wash in water.
7. Counterstain ½ minute with pyronin (0.5% aqueous solution).
8. Wash in water and dry between filter paper.

Anilin-gentian-violet.

Anilin water .....75 c.c.
Sat. alc. sol. gentian violet.....25 c.c.

Anilin water is prepared by adding 2 c.c. of anilin oil to 98 c.c. distilled water, shaking the mixture vigorously for 1 minute and filtering through filter paper until filtrate runs clear.

Gram's Iodine.

Iodine .....1 gram.
Potassium iodide .....2 grams.
Water .....300 c.c.

The object of the iodine is not to stain and not to decolorize, but to act as a mordant which sets the stain in some bacteria so that the alcohol will not decolorize them. Those bacteria in which the stain is set are Gram positive, i. e., they keep the gentian violet stain. Those bacteria which are decolorized by the alcohol are Gram negative and take the counterstain. In pus smears Gram positive bacteria appear dark purple, Gram negative bacteria and leucocytes stain pink.

Ziehl-Neelsen Method. (Stain for tubercle bacillus.)

1. In making smear from sputum, select purulent portion, pick up with wooden toothpick, smear on slide, fix in flame. Cover with carbol fuchsin. Keep steaming hot three minutes.
2. Wash in water.
3. Decolorize in 10% sulphuric acid in 95% alcohol until well spread portions are decolorized.
4. Wash in water.
5. Counterstain ½ minute in Loeffler's Methylene Blue.
6. Wash in water and dry between filter paper.
7. Examine with oil immersion lens. The tubercle bacilli show as slender red rods. Everything else in the sputum stains blue.

Carbol fuchsin.

Phenol crystals, melted.....25 c.c.
Absolute alcohol .....50 c.c.
Fuchsin (basic).....2 grams.

Allow to remain over night in an incubator to insure complete solution, cool and filter. This stock solution is permanent and does not require further filtering. For use, add 1 part of this stock solution to 4 parts of distilled water.

Diagnostic Characters of Pathogenic Bacteria.

Staphylococci.

Morphological and Cultural Characteristics.

The varieties of staphylococci are differentiated on the basis of pathogenicity, pigment formation, liquefaction of gelatin and other cultural properties. They are named from their distinguishing characteristics, as Staphylococcus

pyogenes aureus, Staphylococcus pyogenes albus, Staphylococcus citreus, Staphylococcus epidermidis albus, etc.

Typically the pathogenic staphylococci are Gram positive, appear in smears as spheres in grape-like clusters and as diplococci. Culturally they grow luxuriantly on all media, form moist elevated colonies, white, yellow or orange, depending upon the variety, liquefy gelatin freely, acidify and coagulate milk, and ferment sugars.

Atypically staphylococci may vary in size from small to very large cocci, and may appear as flattened diplococci with a narrow space between the cocci. They may be Gram negative. They may grow in tiny colonies, or in the form of flat colonies with concentric markings, or they may form a film like adherent growth. They may liquefy gelatin very slowly or not at all.

Strains which liquefy gelatin freely are in general more virulent. Staphylococcus pyogenes aureus is more pyogenic than the albus or citreus.

Occurrence.

The staphylococcus is the most common pus producer. It is found most frequently in skin infections as acne pustules, skin abscesses, furuncles, carbuncles. It may cause primary infection of sinuses and may cause septicemia. It is the most common cause of osteomyelitis.

Streptococci and Pneumococci.

Varieties.

No absolute method has been found of differentiating the different varieties of streptococci from one another or from the pneumococcus, but a fairly satisfactory differentiation can be made on the basis of the capsule formation, the behavior on blood agar, the ability to ferment the various sugars, the serum reactions (agglutination and complement fixation), and the pathogenicity towards animals.

DIFFERENTIATING CHARACTERISTICS OF THE PNEUMOCOCCUS AND CERTAIN VARIETIES OF STREPTOCOCCI.

NAME	MORPHOLOGY	CAPSULE	BEHAVIOR ON BLOOD AGAR	FERMENTATION OF SUGARS		PATHOGENICITY
				INULIN	Raffinose	
Pneumococcus	Elongated diplococcus, lancet shaped. Variable in size and shape. May show short chains.	Present	Colony surrounded by greenish zone.	+	+	Very inconstant. Subcutaneous injection of rabbits may produce septicemia or local lesions depending upon the virulence.
Streptococcus pyogenes (hemolyticus)	Chains of round or slightly oval cocci.	Absent	Clear zone of hemolysis about the tiny colony.	O	+	Intravenous injections in rabbits produce arthritis. In large doses myocardial abscesses and hemorrhages.
Streptococcus viridans	Cocci in pairs which resemble pneumococci. Short or long chains of diplostreptococci.	Absent	Green or brown flat colonies surrounded by a zone of green.	O	+	Only slightly pathogenic. Tend to localize in the heart valves, producing endocarditis.
Streptococcus mucosus	Chains of diplococci varying in size and surrounded by a thick capsute which shows no indentation between the pairs.	Present	Colonies transparent, irregular shaped drops of mucoid consistency.	+		Highly pathogenic. Intravenous inoculation produces multiple arthritis. Acute pericarditis and peritonitis may occur.

There are many strains of streptococci found in the human body and its secretions which cannot be readily classified among the streptococci or pneumococci mentioned in the table, partly because of their morphology, partly because of their appearance on blood agar, but chiefly because of their lack of pathogenicity. Some are Gram negative. Some produce dry, brown adherent colonies on blood agar with very slight or no haemolysis or green colorization of the agar and are non-pathogenic towards animals, or at least produce no visible lesion in heart or joints even in enormous doses.

Pathogenicity.

The virulence shown by the streptococci and pneumococci varies considerably. The lesions produced by animal inoculations depend upon the virulence of the strain, the method of inoculation and the number of bacteria inoculated. The most virulent streptococci will cause fatal septicaemia, less virulent strains may cause localized abscesses, erysipelatoid inflammations or endocardial or joint involvement. Streptococcus hemolyticus tends to locate in the joints, while streptococcus viridans tends to locate in the endocardium. The pneumococcus and streptococcus viridans often show little virulence toward animals.

Occurrence.

Found in erysipelas, lymphangitis, cellulitis and puerperal septicaemia, in suppurative inflammatory conditions in joints (as arthritis and acute rheumatism) and serous membranes (as peridicarditis and pleurisy), in otitis media and the throat affections of tonsillitis, diphtheria, scarlatina and measles, in enteritis in infants, and in pneumonia (pneumococcus in lobar pneumonia, streptococcus in the lobular pneumonia usually).

Gram Negative Diplococcus Group.

Gonococcus. (Diplococcus of Neisser.)

Morphological and Cultural Characteristics.

The gonococcus is a coffee bean shaped large diplococcus with flat sides adjacent. It is Gram negative. In pus smears the diplococci appear grouped in the cytoplasm of the pus cells. In freshly infected cases they as a rule are the only bacteria found. In chronic gonorrhea many other bacteria may be present and the microscopic diagnosis is not so simple. The gonococcus grows best on blood agar or ascitic agar in the form of delicate, fine, grayish white colonies. After continued cultivaton it may grow on ordinary media. Isolation of the gonococcus is only possible from acute cases and even then it is very difficult.

Occurrence.

The gonococcus is found in the pus of acute gonorrhea and gonorrheal ophthalmia. Arthritis and endocarditis occur as metastatic complications.

Micrococcus Catarrhalis.—

Morphological and Cultural Characteristics.

The micrococcus catarrhalis has the same morphology in general as the gonococcus, but is often slightly larger and tends to show a sharper outline. In pus smears it generally stains more deeply than the gonococcus and is less often seen within the leucocytes. It grows well on ordinary media forming grayish white or yellowish white often adherent colonies of mortar like consistency.

Occurrence.

Found commonly in secretions of normal and diseased mucous membranes.

Meningococcus.

(Micrococcus intracellularis meningitidis.)

Morphological and Cultural Characteristics.

The meningococcus is a biscuit shaped micrococcus occurring usually in pairs, but may be seen also in fours or masses. Involution forms are common. It is Gram negative and shows some irregularity in staining. In pus smears it is chiefly intracellular. It grows best on neutral ascitic glucose agar, forming flat, grayish white disk like colonies tending to become confluent. It may grow on agar after prolonged cultivation. It tends to die out in a few days after isolation. The different strains vary in the ease with which they can be grown on artificial media, in their fermentative action on sugars, and in their virulence.

Occurrence.

The meningococcus is the most frequent cause of purulent meningitis either sporadic or epidemic. In meningococcus meningitis the spinal fluid is cloudy and contains a large number of polynuclear leucocytes. The meningococcus is found chiefly within the cells.

Bacillus of Tuberculosis.

Morphological Characteristics.

The tubercle bacilli are slender, straight or slightly curved rods often occurring in small clumps, the bacilli lying at an acute angle with one another. They vary in length and often show small nodules or swellings. They are acid fast, that is, they retain the fuchsin stain and are not decolorized by acids or alcohol as the non-acid fast bacteria are. They commonly stain uniformly but may show a beaded appearance due to the deep staining of the nodules and the pale staining of the areas between.

Diagnosis.

Examination of sputum for tubercle bacilli is commonly made. The usual and most satisfactory method is directly by stained smears. Care must be taken to obtain the sputum from the lungs. Purulent portions are smeared on slides, fixed in the flame and stained with carbol fuchsin. (See stains.) Thicker smears may be made in examining for tubercle bacilli than for the usual bacteriological examination, thus permitting one to examine easily more material. One has no difficulty in distinguishing the red stained bacillus even in a thick smear. A careful examination of more than one slide prepared from suspicious purulent portions will show the bacilli if they are present. The tubercle bacilli show as bright red rods against a blue background and show a characteristic arrangement and morphology.

Tubercle bacilli in the urine are much more difficult to find than in sputum. Large amounts of urine must be centrifuged and the sediment smeared on slides, dried, fixed in the flame and stained as sputum smears. The possible presence of other acid fast bacilli as the smegma bacillus rarely makes the diagnosis of tubercle bacilli in the urine uncertain. The examination of a catheterized specimen will usually exclude the smegma bacillus. The only certain method is to inject a guinea pig with the sediment from a large volume of urine.

Diphtheria Group.

(Bacillus diphtheriae and the diphtheroid bacilli.)

Morphological and Cultural Characteristics.

The typical diphtheria bacillus produces a powerful toxin, grows rapidly and grows best on serum media, and shows a characteristic morphology in stained smears. The

diphtheria bacilli are slender rods which vary in length, are often slightly curved, often clubbed or swollen at the end or middle. They may lie at an acute angle with one another or they may lie in palisade arrangement. They are Gram positive. They do not stain uniformly with Loeffler's methylene blue but show granules or barred staining. The pseudo-diphtheria bacilli tend to be short plump rods more uniform in size and shape, and generally do not show polar granules. The true and pseudo-diphtheria bacilli, however, cannot always be differentiated morphologically.

Culturally the diphtheroid bacillus like the diphtheria bacillus grows best on serum media, but may grow well on all media. A valuable means of differentiating the Hofmann bacillus or pseudo-diphtheria bacillus from the diphtheria bacillus is by growing the bacilli in glucose bouillon. The Hofmann bacillus does not produce acid by the fermentation of the glucose while the diphtheria bacillus does. It is not possible, however, to classify the numerous strains of diphtheria and diptheroid bacilli on the basis of their fermentative action on the various sugars. Diphtheroid bacilli of acne and those found in enlarged glands can be isolated anaerobically, but after cultivation may grow aerobically. The acne bacillus is easily and constantly isolated from acne pustules in anaerobic cultures on blood serum.

Pathogenicity.

The virulence of diphtheria bacilli varies considerably, but the majority are of nearly equal virulence. Diphtheria like bacilli are commonly found which are pathogenic to guinea pigs especially in enormous doses, but which produce no diphtheria toxin. Animal inoculation is used as a test of virulence and as a test of toxin production. True diphtheria bacilli cause death in 72 hours of a guinea pig injected subcutaneously with a broth culture in amount

less than 1/5% of the body weight. If a guinea pig injected with antitoxin lives after the injection of about 2 c.c. of the broth culture of a bacillus and the control pig which received only the broth culture dies, one is dealing with a virulent diphtheria bacillus.

Occurrence.

Virulent diptheria bacilli are found in diphtheritic membranes, also occasionally on normal mucous membranes. Diphtheroid bacilli are commonly present on normal and inflamed mucous membranes as of the pharynx, nose, ear, eye (B Xerosis), urethra, vagina, etc., and on the skin as the acne bacillus in acne pustules. They are found in enlarged glands in Hodgkins disease, lymphatic leukemia, leprosy, etc.

Diagnosis of Diphtheria.

The diagnosis of diphtheria often rests upon the bacteriological findings and the early diagnosis is very important. The bacteriological diagnosis can often be made from a smear directly from the throat membrane. This should always be made as it may permit the administration of antitoxin many hours earlier. If the throat smear is doubtful or negative, wait for the examination of the culture. The culture is best made on blood serum and should be examined after 8 to 10 hours incubation as the diphtheria bacillus grows rapidly. Smears from the throat or smears from the culture should be stained with methylene blue. If the diphtheria bacilli are present they will show their characteristic clubbed form or their barred or granule staining.

Hemoglobinophilic Bacillus Group.

Occurrence.

In this group belong the influenza like bacilli found in

some epidemics of influenza, the pertussus bacillus found in whooping cough, the Koch-Weeks bacillus found in acute contagious conjunctivitis, the bacillus of Ducrey found in soft chancre, the influenza like bacilli found in diseases of the respiratory tract, in some cases of acute meningitis, measles and scarlet fever. The bacilli found in these various diseases belong to the group of hemoglobinophilic bacilli and are identical morphologically and culturally, differing only in the matter of virulence and varying somewhat in that.

Morphological and Cultural Characteristics.

All the bacilli of this group require hemoglobin in the culture media for growth. They grow in tiny transparent colonies and die out quickly. They are very small, non-motile, Gram negative, bipolar staining bacilli.

Bacterial Diagnosis.

Differentiation between non-pathogenic influenza like bacilli found in sputum, etc., and this group of hemoglobinophilic bacilli cannot be made in smears. Streak sputum on blood agar. Examine small shining colonies in stained smear to see if there are any influenza like bacilli. Transfer suspicious colonies to plain agar and blood agar. No growth will take place on plain agar if it is a true hemoglobinophilic bacillus. For further identification make agglutination test.

Mucosus Capsulatus Group.

This group includes B. Mucosus capsulatus (Bacillus of Friedlander), B. lactis aerogenes, B. ozenae (Abel bacillus) and probably the Perez bacillus.

Morphological and Cultural Characteristics.

The bacilli of this group are non-motile, Gram negative, generally short broad bacilli but varying in their proportions and usually with prominent capsule. They grow well on all media, and form a viscid mucoid growth, the viscidity depending upon the degree of capsule formation. They generally ferment sugars with acid and gas production. There is a wide variation within the group from the bacilli of the colon type with no capsule to those with prominent capsule formation, from the bacilli growing like B. coli to the bacilli growing in mucus like masses and from the bacilli producing slight or no carbohydrate fermentation to those which ferment all carbohydrates with a large amount of gas production.

Occurrence.

The bacilli of this group are found constantly in the crusts and secretion of atrophic rhinitis and ozena, may be found in diseases of the middle ear and accessory sinuses of the nose, in lobular pneumonia, occasionally in cystitis, pyelitis, pericarditis, pleuritis and meningitis (secondary). B. lactis aerogenes is a normal inhabitant of the intestine especially of children.

Colon-Typhoid Group.

This group includes the colon and paracolon bacilli, the typhoid and paratyphoid bacilli and the group of dysentery bacilli.

Diagnostic Methods.

These consist of (1) the isolation of the bacilli from the blood, feces, urine or other sites of infection, (2) the morphological and cultural study of the bacilli, and (3) for final identification the agglutination test, using immune sera against the various types.

TABLE OF DIFFERENIATING MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF THE COLON-TYPHOID GROUP OF BACILLI.

NAME	MORPHOLOGY.	GELATIN	MILK	SUGARS	INDOL	AGGLUTINATION.	OCCURRENCE
Colon Group	Short rods, sometimes coccus-like, sometimes long threads. Gram negative. Motile.	Not liquefied.	Acidified. Usually Coagulated.	Fermented with acid and gas production.	Usually formed.	Agglutinated by homologous serum in high dilution.	Biliary and urinary tract. Intestinal lesions. Occasionally in pneumonia, meningitis and terminal septicaemia.
Bacillus typhosus	Same morphology. Actively motile.	Not liquefied.	Slightly acidified. Not coagulated.	No gas production.	No indol.	Same	Typhoid fever. Bacilli in blood in first week of disease. Bacilli eliminated in urine and feces.
Bacillus paratyphosus. Type A.	Same morphology. Actively motile.	Not liquefied.	Slightly alkalinized.	Ferments dextrose with gas formation.	No indol.	Same	Paratyphoid fever. Resembles typhoid fever. Found in most cases of paratyphoid fever.
Bacillus paratyphosus. Type B.	Same morphology. Actively motile.	Not liquefied.	Strongly alkalinized.	Ferments dextrose with gas formation.	No indol.	Same	1. Typhoid like type. 2. Gastroenteric type. 3. Cholera like type.
Dysentery Group.	Same morphology. Non-motile.	Not liquefied.	Strongly alkalinized.	Shiga type ferments dextrose only. Four types classified on the basis of sugars fermented.	Indol not formed by shiga type, but is by other types.	Same	Acute dysentery. The bacilli are found in large numbers in the feces in the acute stage of the disease.

Methods of Isolation of the Bacilli from Blood, Urine and Feces.

From blood. Inoculate blood from the vein into broth in the proportion of 1 part of blood to 50 of broth, or into bile media in the proportion of 1 part of blood to 3 of bile. Identify by cultural characteristics and agglutination test with known immune sera.

From urine and feces. To isolate typhoid bacilli, streak plates of Endo medium with centrifuged urine sediment or a suspension of feces made by rubbing up feces with sterile water. By streaking several plates the inoculation can be sufficiently thin to have isolated colonies. Fish translucent dewdrop like colonies and make agglutination test with known typhoid serum.

A probable diagnosis of colon bacilli in urine can easily be made by centrifuging catheterized urine in sterile centrifuge tubes, and adding a few drops of sediment to a fermentation tube of dextrose bouillon. Colon bacilli will ferment the dextrose with gas production

Bacillus Proteus. (Putrefying bacillus.)

Morphological and Cultural Characteristics.

The proteus bacillus is an actively motile Gram negative bacillus varying greatly in size, and ranging from short to long filaments. It grows on all media and best at room temperature, producing a putrefactive odor on blood serum and gelatin with liquefaction of the gelatin. The growth on gelatin plates is characteristic, consisting of an irregular radiating mass of colonies with liquefying center.

Occurrence.

The proteus bacillus is not uncommonly found in pye-

lonephritis and cystitis. Meat poisoning may be due to bacilli of this group. Sometimes associated with other bacteria as pus cocci or alone, the proteus bacillus has been found in purulent peritonitis and phlegmonous inflammations. It may be found in the nasal secretion.

Bacillus Pyocyaneus. (Bacillus of blue and green pus.)

Morphological and Cultural Characteristics.

The pyocyaneus bacillus is very small, slender, actively motile and Gram negative. It grows well on all media, liquefies gelatin, and colors all media bright green in the presence of oxygen.

Occurrence.

The pyocyaneus bacillus may be found in inflammations of the serous membranes as of the pericardial sac and joints, of the mucous membranes as of the ear and sinuses of the nose. It has been found in broncho pneumonia and has been known to cause general infection.

Bacillus Tetani.

Morphological and Cultural Characteristics.

Tetanus bacilli are motile, long slender rods usually single but often in long threads, form large round terminal spores and are Gram positive. They grow on ordinary media only under anaerobic conditions, producing an arborescent growth on solid media. They liquefy gelatin and ferment sugars with gas production. They produce a powerful toxin.

Occurrence.

The tetanus bacillus is found in tetanus through wound infection. The bacilli multiply but little in the animal body and are usually associated with other bacteria, hence it is difficult to isolate them in pure culture. They produce

a powerful toxin at the point of infection, and this spreads to the motor nerves by various channels, causing death.

Diagnostic Methods.

These consist of (1) the examination of smears from the pus of the wound, (2) the study of the cultures made from the pus or infecting material and (3) animal inoculation.

1. Make smears from the pus of the wound, stain with Gram, and examine for spored bacilli; the tetanus bacillus is seldom found in the pus.

2. Inoculate pus or bits of tissue or foreign bodies found in the wound into glucose bouillon and make anaerobic. Incubate the cultures 24 to 48 hours, then heat them ½ hour at 80° C. to kill all vegetative forms of bacteria. Inoculate the heated culture into glucose bouillon or milk, make anaerobic, incubate 24 hours and examine for the tetanus bacillus.

3. To make certain test, inoculate mice or guinea pig subcutaneously with salt solution emulsion of material from the wound. Tetanus will result in 1 to 4 days if the tetanus bacilli or spores are present.

Bacillus Anthracis.

Morphological and Cultural Characteristics.

Anthrax bacilli are large, non-motile rods with square cut ends. They grow in long chains, often in twisted bundles and show a prominent capsule. They are Gram positive. They form spores only in the presence of oxygen, hence not in the animal body. They grow on all media and liquefy gelatin. Their colonies are very characteristic and consist of a tangled mass of filaments.

Occurrence.

The anthrax bacillus is the causative agent in malignant pustule and intestinal and pulmonary anthrax. They may

be recognized in smears from the fluid of the malignant pustule, in the feces from intestinal anthrax and in the sputum from pulmonary anthrax. The diagnosis may be established by cultural study and animal inoculation, the anthrax bacillus causing death of guinea pig by septicaemia.

Bacillus Aerogenes Capsulatus. (B. Welchi.)

Morphological and Cultural Characteristics.

The B. Welchi is a large non-motile bacillus growing singly and in chains. It forms a capsule when growing in the body and spores occasionally in cultures. It is Gram positive. Culturally it is a strict anaerobe and grows well on all media. It ferments the sugars with energetic gas production. It produces a characteristic growth (stormy fermentation) in milk, that is, it forms a much riddled clot because of the abundant gas formation. The milk is acidified by the formation of butyric acid which gives its characteristic odor to the culture.

Occurrence.

B. Welchi is common in the intestine and soil. It is the cause of emphysematous gangrene.

Pathogenic Trichomycetes.

Microorganisms of this group of higher bacteria grow in the form of long threads varying in length and thickness, some showing club like terminations, some branching. Among them there are Gram positive and Gram negative varieties, acid fast and non-acid fast varieties, aerobic and anaerobic varieties, some growing readily in artificial media, some grown with great difficulty. The growth on solid media is generally granular, adherent, dry like a mould, and in liquid media white, thistledown like tufts of interlacing filaments, or they may form a pellicle. They are widely distributed and not infrequently met with. They are of a low grade of pathogenicity, the usual reaction to

infection in man and animals being chronic granulation tumors with or without suppurative foci.

Varieties.—

Leptothrix. This grows in long, straight, thread like form and shows no branching. It is usually Gram negative. It is found frequently in the human mouth about the teeth and tonsils. It is of doubtful pathogenicity.

Nocardia (*Streptothrix*). This grows in branching thread like form and in smears appears as a tangled mass of threads. There are Gram positive and Gram negative strains. The growth is usually like that of a mould, but the different strains vary much in the ease with which they grow on artificial media. The anaerobic varieties generally show a scanty growth as compared with the aerobic.

It may be found in skin abscesses, alveolar abscesses, brain abscesses, cerebrospinal meningitis, pneumonic areas, pseudotuberculosis of the lungs, etc.

Actinomyces. This grows as branching threads. It is typically anaerobic. In the tissues the actinomyces grows in colonies in the form of yellow granules of about pin-head size. For diagnosis examine the pus or granulation tissue for these yellow granules. Crush the granules between slides and examine unstained with high dry lens, also stain with Gram and examine with the oil immersion lens. The granules will be seen to be made up of a tangled mass of filaments tending to radiate from a center and showing characteristic club like terminations. The center of the colony may have a mass of coccus like bodies or conidia. The filaments and spores are Gram positive and the clubs usually Gram negative.

Actinomyces causes the disease actinomycosis, which is rare in man. It may occur primarily in the mouth, head or neck, on the skin, in the lungs and in the intestine.

Bacteriology of Conjunctival Secretions.

The diagnosis of the bacteria in conjunctivitis can often be made by the examination of a Gram stained smear from the conjunctival secretion. In making a smear the discharge should be taken from the conjunctival surface avoiding contaminations from the lid margins, etc. If the discharge is slight it is best to obtain what has collected at the inner canthus of the eye. Sometimes gentle pressure upward along the lachrymal duct will bring forth some discharge into the inner canthus of the eye. For collection of the discharge it is convenient to use wooden applicators wound at the tip with a bit of cotton and sterilized. A platinum loop may also be used. Smears are made on clean slides by rubbing the pus collected on the cotton tip of the applicator or platinum loop into a thin layer on the slide. The smear is then stained with Gram's stain and examined with oil immersion lens.

Koch-Weeks Bacillus. This may be found in acute contagious conjunctivitis. The bacilli are Gram negative slender rods of varying length. Morphologically they cannot be distinguished from the influenza bacillus.

Bacillus Influenzae. This may be found in catarrhal conjunctivitis. The bacilli are Gram negative very short rods, often resembling elongated diplococci.

Morax-Axenfeld Bacillus. This may be found in subacute conjunctivitis. The bacilli are large Gram negative diplobacilli, some appearing in short chains.

Bacillus Xerosis. This may be found on the normal conjunctiva. The bacilli are Gram positive diphtheroid bacilli.

Pneumococcus. This may be found in acute catarrhal conjunctivitis. The pneumococci are Gram positive, elongated often lancet shaped diplococci. The capsules are not seen in eye smears.

Gonococcus. This may be found in purulent conjunctivitis. The gonococcus is a Gram negative biscuit shaped diplococcus, chiefly intracellular.

Micrococcus Catarrhalis. This may be found occasionally on the normal conjunctiva and in simple catarrh. It is a Gram negative biscuit shaped diplococcus, generally extracellular and often in clusters. Generally the secretion is scanty and poor in cellular elements in proportion to the number of cocci.

Meningococcus. This may be found in conjunctivitis accompanying meningococcal meningitis. The meningococcus is a Gram negative biscuit shaped diplococcus, chiefly intracellular.

Streptococcus. This may be found in severe membranous conjunctivitis. The streptococci are Gram positive spherical diplococci and in chains.

Bacteriology of Vincent's Angina.

The diagnosis is easily and quickly made by examination of a smear from the exudate or membrane in the throat. The smear may be stained with methylene blue or Gram's stain. Spirilla and fusiform bacilli in large numbers, and a moderate number of leucocytes will be found in the smears. The spirilla are Gram negative, small and show shallow spirals. The bacilli are Gram negative, irregularly stained with methylene blue, are long, slender and spindle shaped.

Cultures from the exudate on blood serum under anaerobic conditions may yield a growth of the fusiform bacillus mixed with other bacteria. Such cultures have a foul odor.

Preparation of Bacterial Vaccines.

The preparation of an autovaccine consists in, (1) the obtaining of a bacterial culture, (2) the making of an emulsion in salt solution of this culture, (3) the sterilizing of the emulsion, (4) the counting of the bacteria in the

emulsion, and the diluting and bottling of the vaccine ready for use.

Cultures.—

The most difficult and most important step in the making of vaccines is the obtaining of satisfactory cultures. It is always advisable to first examine the smears made directly from the specimen, and to use this information about the varieties and numbers of bacteria present as a basis in selecting proper media and determining the amount of the material to be used in inoculation. To obtain cultures from abscesses, pustules or other pus containing a single organism, platinum loopfuls of the pus are smeared over the surface of 4 or 5 tubes of Loeffler's blood serum or plain agar, and incubated 24 hours. If there is a mixture of bacteria in the specimen of pus, sputum, urine, feces, tissue, etc., from which the vaccine is to be prepared it will be necessary to separate the varieties of bacteria and get them in pure culture. To do this the material should be streaked over the surface of the media in Petri dishes of blood agar, over slant surface of a series of tubes of blood serum, and inoculated into liquid media as ascitic glucose bouillon, some of the inoculations being rendered anaerobic. Pure cultures from the different bacteria can then be obtained from the single colonies and subcultures used in the preparation of the vaccine. Solid media, especially blood serum and blood agar, (5 to 10 tubes) is usually most practical for making the cultures for the vaccine, but in the case of streptococci and pneumococci more growth can be obtained in inoculation of about 50 c.c. of glucose bouillon or ascitic glucose bouillon.

Preparation of the Emulsion.—

If the cultures are on solid media add a few c.c. of sterile salt solution to each tube and gently scrape the growth from the surface with a platinum loop mixing with the solution. Pour the bacterial emulsion from each tube

into a sterile centrifuge tube, centrifuge 2 or 3 minutes and pour off turbid supernatant fluid into sterile 25 c.c. bottle. Add a few c.c. of sterile salt solution to sediment in centrifuge tube, mix thoroughly by shaking, repeat centrifuging and again pour off supernatant cloudy fluid into same bottle with previous emulsion. This process can be repeated if necessary in order to get into an emulsion nearly all of the sediment, but usually twice is sufficient. This method gives a uniformly cloudy fluid, removes any clumps of bacteria and avoids the labor of prolonged shaking to break up the clumps.

If the culture is in bouillon, this must be poured into sterile centrifuge tubes and centrifuged. The supernatant bouillon is then poured off and discarded, and sterile salt solution added in its place. The bacterial sediment is mixed well with the salt solution, the tube centrifuged and the supernatant fluid poured into a sterile bottle, the process being repeated if necessary until most of the bacterial sediment is in the form of a uniformly turbid emulsion.

Sterilizing the Vaccine.—

Sterilize the bacterial emulsion by keeping it at a temperature of 60° C. for one hour. This is done by placing the bottle containing the emulsion in a water bath at 60° C. allowing the water to come well up on the neck of the bottle. Any bacteria on the inside of the neck of the bottle are first killed by thoroughly flaming the neck of the bottle with Bunsen flame.

Counting the Bacteria in the Emulsion.—

Pour a few drops of the sterilized bacterial emulsion into a watch crystal. Have ready a clean dry watch crystal, a watch crystal with normal salt solution, three clean slides which have been passed slowly through the Bunsen flame 4 or 5 times to burn off grease, one capillary glass pipette with rubber teat fitted over the large end, and a few strips of cigarette paper cut the width of a slide.

Mark the glass pipette with a glass pencil one inch from the tip. Stab finger with a needle and press out drop of blood. By suction with rubber teat draw up blood to pencil mark, draw in small measure of air then same measure of bacterial emulsion as blood, followed by 8 measures of salt solution each separated by air. Press all out in dry watch crystal, mix by drawing back and forth through pipette, then place a drop of the mixture near the end of each of the three slides. Make a thin smear by touching cigarette paper to the drop and pulling paper gently along the slide. Dry smear in the air, fix in 7% mercuric chloride for one minute, wash in water and stain in alkaline methylene blue 5 minutes.

To count the bacteria examine the smears with oil immersion lens, counting the red blood cells and the bacteria in about 25 fields on each slide, usually a total of about 300 or 400 red cells. Put the number of red cells counted in each field in one column and the number of bacteria counted in each corresponding field in another column, and sum up each separately. A small circle about ½ inch in diameter drawn on the lower glass of the eye piece of the microscope with a pen or glass pencil forms a convenient small field in which all the cells can be easily and quickly counted. Well and evenly spread portions of the smears should be used in counting.

To calculate the number of bacteria per c.c. in the emulsion the following proportion is determined, it being known there are 5 million red cells per cmm. of blood.

$$\frac{\text{Number of red cells counted}}{\text{Number of bacteria counted}} = \frac{5 \text{ million}}{x}$$

x= number of bacteria per cmm. multiplied by 1000= number of bacteria per c.c.

Diluting and Bottling the Vaccine.—

Bacterial vaccines of staphylococci are usually diluted to contain 1000 million bacteria per c.c., streptococci 200 mil-

lion per c.c., and most bacilli 500 million per c.c. In diluting calculate the total number of bacteria which will be present in the vaccine; for example there will be 25,000 million staphylococci in a vaccine bottle containing 25 c.c. of vaccine with 1000 million bacteria per c.c. If the bacterial emulsion as prepared contains 5000 million bacteria per c.c., 5 c.c. of this emulsion must be added to 20 c.c. of salt solution in order to have 25 c.c. of vaccine with 1000 million bacteria per c.c.

It is convenient to have on hand amber bottles containing 25 c.c. of normal salt solution cotton plugged and sterilized in the autoclav. Use a glass Luer syringe (10 c.c.) in making the dilution, sterilizing the syringe by drawing ether back and forth through the needle and the syringe a few times. Having calculated the number of c.c. of the bacterial emulsion required in the complete vaccine, that number of c.c. of salt solution is withdrawn from a bottle containing 25 c.c. with the sterile syringe and replaced by a corresponding number of c.c. of the emulsion. Add as antiseptic ½ c.c. of a 5% solution of carbolic acid to the 25 c.c. of vaccine. Fit rubber top previously immersed in 7% mercuric chloride for 5 minutes on bottle and place rubber band tightly just below rim of bottle. It is practical to use heavy surgeon's rubber finger cots, cutting off the finger portion so as to leave about 1 inch of tip.

To determine the sterility of the vaccine, shake the bottle, touch rubber top with lysol, withdraw from inverted bottle about ½ c.c. of vaccine into sterile Luer syringe. Inoculate this into a tube of agar or blood serum and incubate 24 hours. If the vaccine has been prepared from an anaerobic organism requiring special media for growth, use the same conditions in planting out vaccine as were necessary in growing the bacteria in the first place.

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No. Patient Date

Physician

BLOOD EXAMINATION

Haemoglobin

Erythrocyte Count

Leucocyte Count

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STAINED FILM

DIFFERENTIAL LEUCOCYTE COUNT	ERYTHROCYTES
Polymorphonuclear Neutrophiles	Poikilocytosis
Small Mononuclears	Anisocytosis
Large Mononuclears	Polychromatophilia
Transitionals	Nucleated Reds Per 100 Leucocytes
Eosinophiles	Normoblasts
Basophiles	Megaloblasts
Myelocytes	Microblasts

Parasites

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Wassermann

Complement Fixation Test for Gonorrhoea

FORM FOR REPORT BLANKS

No. Patient Date

Physician

URINALYSIS

QUALITATIVE	QUANTITATIVE
Color	Quantity in 24 Hours
Reaction	Total Acidity
Specific Gravity	Total Solids
Albumin	Quantity
Sugar	Quantity
Indican	Urea
Bile	Uric Acid
Blood	Chlorides
Acetone	Sulphates
Diacetic Acid	Phosphates
Diazo Reaction	Ammonia
Functional Kidney Test	

MICROSCOPIC:

Casts

Cylindroids

Blood

Pus

Crystals

Amorphous Deposits

Epithelial Cells

FORM FOR REPORT BLANKS

No. Patient Date

Physician

EXAMINATION OF STOMACH CONTENTS

Test Meal Withdrawn in Hrs.

Quantity Rel. Amt. Liquid

Odor Food Particles

Color Mucus

CHEMICAL

Total Acidity Starch

Free HCL Erythrodextrin

Combined HCL Acchroodextrin

Free Acids and Salts Peptone

Organic Acids and Salts Bile

Lactic Acid Blood

Ferments Pus

MICROSCOPIC

Bacteria Pus

Yeast Blood

Sarcinae Epithelium

Oppler Boas B Mucosa

Food Remnants From Former Meals

FORM FOR REPORT BLANKS

No. Patient Date

Physician

EXAMINATION OF FECES

Color

Consistency

Reaction

Undigested Food

Connective Tissue

Stones

Fat

Blood

Bile

MICROSCOPIC:

Muscle Fibre

Free Starch Granules

Neutral Fat

Crystals

Pus

Parasites

FORM FOR REPORT BLANKS

No. Patient Date

Physician

CEREBROSP NAL FLUID

Amount

Appearance

Total Cell Count

Differential Cell Count

Globulin Tests

Fehling's Solution Reduction

Lange's Colloidal Gold Test

Wassermann

Bacteriological Examination

Stained Smears from Sediment

Cultures

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